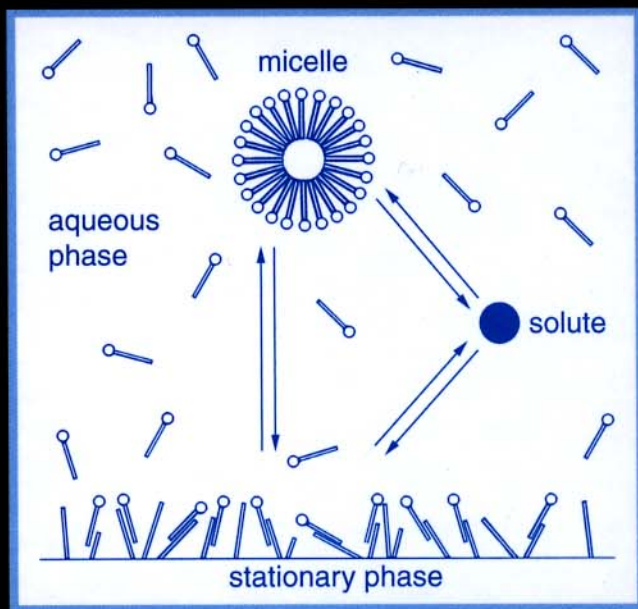


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Micellar Liquid Chromatography



Alain Berthod
Celia García-Alvarez-Coque

Micellar Liquid Chromatography

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Modern Chromatographic Analysis of Vitamins, Third Edition, Revised and Expanded, *edited by André P. De Leenheer, Willy E. Lambert, and Jan F. Van Bocxlaer*

Micellar Liquid Chromatography

Alain Berthod

*Université Claude Bernard, Lyon I,
Villeurbanne, France*

Celia García-Alvarez-Coque

*Universidad de Valencia,
Burjassot (Valencia), Spain*



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The MICHROM software was written exclusively for this volume by José Ramón Torres-Lapasió, University of Pamplona, Spain.

*A Guillermo, mi esposo y compañero de trabajo, por su ayuda y apoyo,
A Sergio y Sonia, mis hijos, por su agradable compañía,
A mis buenos amigos que hicieron posible este libro.*
C.G.A.C.

*A mon épouse, Carmen, pour sa patience et la liberté qu'elle me laisse,
A mes filles, Magali et Laurence,
restez jeunes mais utilisez votre tête et votre bon sens,
A ma famille, belle-famille et aux amis,
incroyable, mais oui, parfois les chercheurs trouvent. . .*
A.B.

Foreword

When studying micelle-catalyzed peptide and oligonucleotide formation in late 1974 and 1975, I had no idea that this research would eventually lead to a new branch of separation science. In the course of these synthetic studies, we had to separate a variety of products produced in the oligomerization reactions. We subjected the solution containing our products to traditional liquid chromatography on columns up to two meters in length. We noticed that the elution behavior of the standards we used was completely different when we chromatographed the standards alone as compared to the spiked reaction solution. It soon became clear that the micellar "catalyst" in the reaction solution was altering the separation. Soon afterwards we found that we could actually use some forms of chromatography to measure the binding constants of various molecules to micelles. In retrospect, my decision to try using micellar solutions as mobile phases in LC was a logical progression of this work, but at that time, the deliberate use of micelles in analytical chemistry was unheard of and was met with a good deal of skepticism. The first paper on the deliberate use of micelles as a mobile phase for LC was published in 1977. This was quickly followed by successful applications in TLC and HPLC and a theoretical treatment based on the micellar pseudophase. Micellar liquid chromatography (MLC) was about to become a novel part of the great HPLC boom that occurred from the late 1970s through the 1980s.

In early July 1980, a session devoted to the analytical applications of micelles was given as part of the International Symposium on *Solution Behavior of Surfactants* (in Potsdam, NY). It was a huge success, often

with standing-room-only crowds. The 1981 Gordon Conference on Catalysis in Micellar and Macromolecular Systems (Wolfboro, NH) also devoted a session to micelles in chemical analysis. The co-organizer of that session was Willie L. Hinze, who first demonstrated the use of micelles in enhancing spectroscopic analysis. Also, by that time we were using cyclodextrins as beneficial pseudo-phases in separations. At about this same time, the use of separation-based techniques to measure association constants to micelles, cyclodextrins, etc. was also popularized.

A large number of scientists began investigations involving micelles in separations. By the mid-1980s, the journal *Analytical Chemistry* had established a separate review category for micellar liquid chromatography. It is interesting to note that our work on MLC led directly to the use of cyclodextrins in chromatography (and the "chiral separations revolution") as well as to their use in capillary electrophoresis. The authors of this book are among the leading European scientists in the area of micellar separations research. Both have made significant contributions to the field. Early on I was fortunate to meet Alain Berthod because of his work in MLC. As a result, we have successfully collaborated on many projects for over one and a half decades.

Looking back, two things are most gratifying about our development of micelle-based separations. The first is that micellar methods are widely used and have solved many problems for scientists and technicians. Second, I have had the opportunity to meet and interact with many wonderful people all over the world as a result of our joint interest in micellar or pseudo-phase-based separations.

This is a particularly opportune time for this book. The field is sufficiently mature that one is able to examine it with a proper perspective and to see how it gave rise and spread into other areas of separation science and analytical chemistry. *Micellar Liquid Chromatography* is a thorough scholarly and practical presentation of all areas of this special separation technique. It is likely that it will be the definitive reference volume in this area of research and technology for years to come.

Daniel W. Armstrong

Curator Professor

Head of the Analytical Division

Department of Chemistry

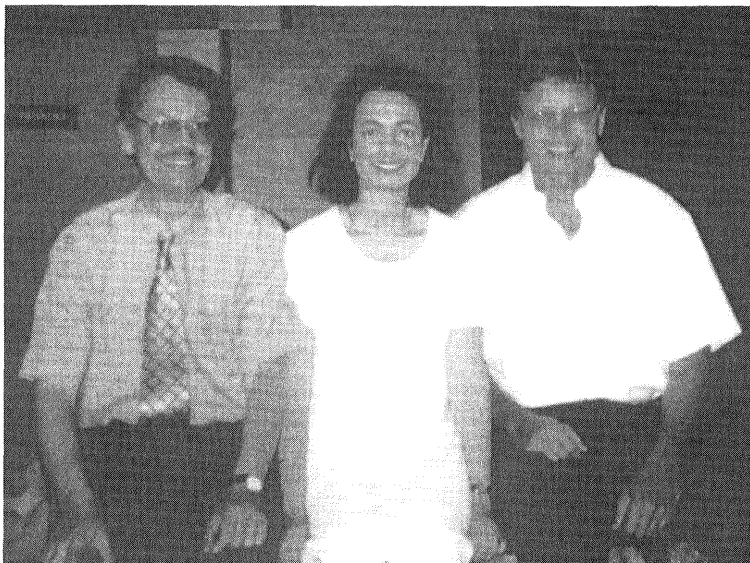
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Preface

Our main concern in starting this almost three-year work was to fill a gap: there was no dedicated book exploring thoroughly the theme of Micellar Liquid Chromatography (MLC). Several reviews appeared in different journals. Chapters dealt with MLC in different analytical chemistry books. However, it was necessary to have a handbook exclusively dedicated to the MLC technique. This is the goal of this book. We tried to collect as much information as possible on the topic, adding the chemical knowledge needed to develop it. We tried to scan exhaustively the literature for MLC articles up to early 1999. We chose to cite every reference with its full title so that the reader has a good idea of the covered topic.

We wrote for the specialist, but we tried to explain the difficult points starting from the basics. We shared the work equally. One of us, being interested in the physicochemistry of the micellar media, the role of the stationary phase and the efficiency problem, prepared the chapters on these topics. The other, being an expert in micellar partitioning and modeling and optimization of retention, wrote the corresponding chapters. We corrected each other's work to obtain some homogeneity.

We think that the text will be useful for people wanting to start MLC analyses and also for people working in related fields such as the separation methods using micelles and capillary electrophoresis or electrochromatography. Students specializing in analytical chemistry will find useful information as well.



From left to right, Alain Berthod, Celia García Alvarez-Coque and Daniel W. Armstrong at HPLC'99 in Granada (Spain), June 1999.

We would like to thank first Jack Cazes and Russell Dekker, who invited us to prepare this book and published it after waiting patiently throughout the writing process. During the progress of the book, we realized that the exposed algorithms, needed to optimize new applications, were difficult to use. We then asked Professor Jose Ramón Torres-Lapasió to develop software to facilitate the mathematical treatment. This gave birth to MICHROM, a software program included on a CD-ROM with this book. We cannot thank him enough for this great enhancement of our work. We thank Christelle Garon-Boucher and Laurent Veyre for scanning the literature for cmc and micellar partition coefficient values that allowed us to prepare the important lists of such data included as Appendices. We thank also Joe P. Foley for reviewing our chapter on modeling. We conclude this preface thanking the inventor of the MLC technique, Daniel W. Armstrong, for his continuous support for more than twelve years.

Lyon (France) and Valencia (Spain), October 1999

Alain Berthod and Celia García Alvarez-Coque

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Symbols and Abbreviations

A'	first constant of the Knox equation (flow anisotropy)
$[A]$	concentration of free solute in bulk water
$[AM]$	concentration of solute associated to the micelle
B'	second constant of the Knox equation (longitudinal diffusion)
B/A	asymmetry factor measured at $W_{0.1H}$
C'	third constant of the Knox equation (mass transfer)
C_{aq}^-	concentration of an added salt to a micellar solution
C_d	longitudinal diffusion contribution to plate height
C_e	eddy diffusion contribution to plate height
CF	correlation factor for PAH calculated as [(number of double bonds) + (number of primary and secondary carbon atoms) - 0.5 for a nonaromatic ring]
C_m	mass transfer contribution to plate height
$C_{n_{OH}}$	alkyl chain length of a linear alcohol
$C_{n_{surf}}$	alkyl chain length of the tail of a surfactant molecule
C_s	stationary phase mass transfer contribution to plate height
C_{sm}	stagnant mobile phase mass transfer contribution to plate height
D_m	solute diffusion coefficient in the mobile phase
D_s	solute diffusion coefficient in the stationary phase
d_p	stationary phase particle diameter
F	Fisher coefficient

f_m	fraction of mobile phase inaccessible to the solute
f_s	fraction of stationary phase unavailable to the solute
ΔG_c	free energy of cavity formation
ΔG°	Gibbs free energy of reaction
H_0	height at the peak maximum
ΔH°	standard enthalpy of transfer from mobile phase to stationary phase
IGC50	50% inhibitory growth concentration of phenols in the culture of <i>Tetrahymena pyriformis</i>
k	retention factor (previously called capacity factor and noted k')
k_0	retention factor at zero micelle concentration
K_{AD}	relative variation in the concentration of solute in bulk water upon addition of a modifier
K_{AM}	solute-micelle equilibrium constant
K_{AS}	partition constant between stationary phase and water multiplied by the phase ratio
K_D	GPC constant solute size depending
k_{calc}	calculated or predicted retention factor
k_{exp}	experimental retention factor
K_{ieM}	constant of the ion-exchange equilibrium at the micelle-solution interface
K_{ieS}	constant of the ion-exchange equilibrium at the stationary phase-solution interface
K_{MD}	relative variation in the concentration of solute in micelle upon addition of a modifier
K_S	autoprotolysis constant of water ($= 10^{-14}$ at 20°C)
K_{SD}	relative variation in the concentration of solute in the stationary phase upon addition of a modifier
k_w	retention factor with a mobile phase of 100% water
K_{WM}	micellar partition coefficient or solute-micelle distribution constant
K_{WS}	solute-stationary phase equilibrium constant
L	average distance of a molecule from the wall of a FFF channel

L/B	ratio of the maximalized length-to-breadth of the rectangle enclosing the molecules
$\log K_H$	protonation constant
M	methionine
[M]	surfactant forming micelles (total concentration of surfactant minus cmc)
$M_{W/AOT}$	number of water molecules per AOT surfactant molecule
N	efficiency or plate number
N	micelle aggregation number (number of surfactant molecules in one micelle)
n_C	number of carbon atoms in an homologue compound
P_{ow}	1-octanol-water partition coefficient
P_{SM}	partition coefficient between stationary phase and micelles ($=P_{WS}/P_{WM}$)
P_{WM}	partition coefficient between water and micelles per surfactant molecule
P_{WS}	partition coefficient between water and stationary phase
pK_a	$-\log$ of the acid dissociation constant K_a
Q_{cmc}	amount of adsorbed surfactant
QH	quantitation of hydrophobicity index
R	constant of perfect gases
R_s	resolution factor
[S]	concentration of a solute in a micellar medium ($= [A] + [AM]$)
S	elution strength parameter in hydro-organic mobile phases
S_{hyb}	elution strength parameter in hybrid micellar systems
ΔS	entropy variation
ΔS°	standard entropy of transfer from mobile phase to stationary phase
SP	solvent-related properties of solutes
T	absolute temperature
t_D	delay time (time before the gradient reaches the top of the column)
t_{eo}	retention time of a neutral solute moving with the electro-osmotic flow in CE
t_g	gradient retention time

t_{mc}	retention time of a micelle in MEKC
t_o	dead time
t_R	time at the peak maximum
u	mobile phase linear velocity
V_e	total volume of eluent needed to elute a given solute from the column
V_i	pore volume in GPC stationary phase
V_o	column dead volume
V_R	solute retention volume ($= V_e$)
V_S	volume of the active surface of the stationary phase
W	width of a FFF channel
W	tryptophan
$W_{0.1H}$	width of the peak at 10% of peak height
Y	tyrosine

Greek Letters

α	chromatographic selectivity (k_2/k_1 , 2 is the most retained solute)
$\alpha(CH_2)$	methylene selectivity
$\alpha(CO)$	selectivity of a carbonyl group
α_{AM}	binding selectivity to micelles
$\alpha_{i,i+1}$	separation factor or selectivity between solute i and solute $i+1$
α_{WS}	stationary-phase partitioning selectivity
β_{HL}	complexation constant of a metallic ion with a H_2L complexing agent
β_L	complexation constant of a metallic ion with a HL complexing agent
γ_m	obstruction factor in stagnant mobile phase
γ_s	obstruction factor in porous or granular material
δ	degree of counterion binding to micelles
ρ	mole percentage of solute in the micelles
φ	concentration of modifier

ϕ_m	organic volume percentage in a micellar solution or microemulsion
Φ	phase ratio ($= V_s/V_o$)
ϕ_f	thickness of the stationary phase useful layer
μ	total concentration of surfactant
v	partial specific volume of the monomers of surfactant in the micelle
π_{R1}	$\log P_{ow}$ for the R_1 substituent
σ_G	standard deviation linked to peak width
σ^2	chromatographic variance
v	reduced mobile phase velocity ($= u_d/p/D_m$)
Ψ	stagnant mobile phase fraction

Acronyms

ACN	acetonitrile
AES	atomic emission spectrometry
AOT	sodium diethyl hexyl sulfosuccinate (also called Aerosol OT®)
AY	alanyl-tyrosine
BET	Brunauer, Emmett and Teller method (using gas adsorption to measure the surface area of a porous material)
BSA	bovine serum albumin
CE	capillary electrophoresis
cmc	critical micelle concentration (mol/L)
CMX	cefmenoxime hemihydrochloride
CTM	cefotiam dihydrochloride
CTAB	cetyltrimethylammonium or hexadecyltrimethylammonium bromide
C_{14} TAB	tetradecyltrimethylammonium bromide
CTAC	hexadecyltrimethylammonium chloride
DDTC	diethyldithiocarbamate
DF	aspartyl-phenylalanine
DMA	dimethylarsenic acid

DTAB	dodecyltrimethylammonium bromide
DTC	dithiocarbamate
EAQ	ethyl anthraquinone
EC	electrochromatography
ELSD	evaporative light scattering detector
FID	flame ionization detector
FF	phenylalanyl-phenylalanine
FFF	field flow fractionation
GLY	glycyl-leucyl-tyrosine
GPC	gel permeation chromatography (also called size exclusion chromatography)
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HETP	height equivalent to a theoretical plate
HPLC	high-performance liquid chromatography
IAHQ-Glu	5,8-dideaza-isopteroyl- γ -glutamyl-L-glutamic acid
ICP-MS	inductively coupled plasma mass spectrometry
IOC	International Olympic Committee
ISRP	internal surface reversed-phase
LOD	limit of detection
LSE	linear solvation energy relationship
LY	leucyl-tyrosine
LW	leucyl-tryptophan
MD-HGH	methionylaspartyl-human growth hormone
MEKC	micellar electrokinetic chromatography
MH	maleic acid
MLC	Micellar liquid chromatography
MMA	monomethylarsonic acid
MS-RTP	micelle-stabilized room temperature phosphorimetry
NAC	N-acetyl-L-cysteine
NED	N-(1-naphthyl)ethylenediamine dihydrochloride
NMR	nuclear magnetic resonance
ODS	octadecyl-bonded silica
OPA	o-phthalaldehyde

O/W	oil in water emulsion or microemulsion (aqueous continuous phase)
PAH	polycyclic aromatic hydrocarbon
QRAR	quantitative retention-activity relationship
QSAR	quantitative structure-activity relationship
QSRR	quantitative structure-retention relationship
RPLC	Reversed-Phase Liquid Chromatography
SDS	sodium dodecyl sulfate (sodium lauryl sulfate)
SFC	supercritical fluid chromatography
THPA	tetraheptylammonium bromide
TLC	thin layer chromatography
W/O	water in oil emulsion or microemulsion (organic continuous phase)

Micellar Liquid Chromatography

1

Presentation of the Book

I. HOW THE AUTHORS BECAME INVOLVED IN MICELLAR LIQUID CHROMATOGRAPHY

In the late seventies, Dr. Berthod investigated the physicochemical properties of micellar solutions and microemulsions by using electrochemistry [1, 2]. He was also involved in liquid chromatography (LC). After reading the early work of Armstrong on micellar liquid chromatography (MLC) [3, 4], Berthod decided to study the modifications of the stationary phase of a chromatographic column exposed to micellar solutions [5, 6]. The results of this study were evaluated by Armstrong himself. This was the starting point of a very fruitful scientific collaboration, which began with the writing of a concerted article on the diffusion coefficient of solutes in micellar solutions [7]. Armstrong and Hinze invited Berthod –at the 1986 ACS National Symposium in New York –to the special session titled *Ordered Media on Chemical Separations* [8], that they were organizing. There, Berthod met most of the researchers involved in MLC at that time: Armstrong, Cline-Love, Dorsey, Fendler, Hinze, Mullins, Pramauro, Pelizzetti and Sybilska.

In June 1986, Berthod started a sabbatical stay at the Department of Chemistry at the University of Florida with Prof. J. D. Winefordner to study the hyphenation of laser spectroscopy with LC [9]. In December 1986, Dr. García-Alvarez-Coque joined Winefordner's research group to investigate room-temperature phosphorescence in micellar media [10]. The exchange of ideas between both Berthod and García Alvarez-Coque inspired their collaboration on fluorescence in microemulsions and reversed micelles [11].

Upon returning to Europe, Berthod began work on the reduced efficiency problem in MLC [12]. García-Alvarez-Coque began work also, on the improvement of derivatization reactions of organic compounds by the use of micellar media [13]. In the early nineties, Dr. Laserna, another member of Winefordner's group, involved both Berthod and García-Alvarez-Coque in the analysis of drugs used illegally in sport [14, 15]. At this time, García-Alvarez-Coque also began to work on MLC. She wrote a review on the solute-micelle and solute-stationary phase interactions [16]. Afterwards, García-Alvarez-Coque became very interested in modeling the retention behavior of solutes [17, 18]. Together with Dr. Torres Lapasió, they prepared the software program *MICHRON* for the development of MLC applications, that is included with this book.

II. THE TECHNIQUE

Micellar liquid chromatography is an alternative to conventional reversed-phase liquid chromatography (RPLC) with aqueous-organic mobile phases. It joins the advantages of micellar media with the separation capability of LC. The mobile phases are aqueous solutions of a surfactant at a concentration above the critical micellar concentration (cmc), that is, in a medium where micelles exist. The variety of possible interactions between solutes, micelles and stationary phase gives a large versatility to this technique and makes it appropriate for a wide range of solute analyses. Mixtures of hydrophilic and hydrophobic compounds can be separated in one run. This adaptability is perhaps the most important characteristic of MLC.

Another advantage of the use of micellar solutions as mobile phases is the solubilization of nonpolar molecules. The necessary low amount of organic solvent used in micellar phases is very positive. It reduces the toxicity, flammability, environmental impact and cost of these phases.

Micellar mobile phases have posed, however, some serious problems, that have slowed the development and widespread use of MLC. First, the chromatographic efficiency is often much lower in MLC than that

observed with similar columns in conventional RPLC. Secondly, the eluent strength of micellar solutions can be extremely weak. Fortunately, the addition of an organic modifier, such as an alcohol, can greatly remediate both of these problems.

The position (retention) and shape (efficiency) of the chromatographic peaks depends on the nature and concentration of the surfactant and modifier, but it can also depend on the mobile phase pH, temperature and ionic strength. All these parameters can be modeled and optimized to provide an adequate separation of a mixture of solutes.

The main use of the technique is in the analysis of physiological fluids. Micellar mobile phases are able to maintain proteins in solution. They allow the direct injection of biological samples in MLC systems. The protocol of the analysis is dramatically simplified.

III. THE BOOK

The pioneering work of Armstrong and the early research performed in MLC focused mainly on the study of the retention mechanisms and principles. Although some interesting applications were also reported from the beginning, most of the applied work has been done in recent years. The technique has reached maturity, and needs a reference organizing the information given by the more than three hundred papers, published to date.

It can be thought that working on MLC is just mixing the surfactant with water, and flushing the solution through the column. As with any other technique, working without a minimum knowledge will only produce disappointing or misleading results. The use of micellar solutions, without special care, can easily damage the column and even the chromatographic system. One of the purposes of this book is to expose the procedure to be followed and the reasons for the problems that can develop.

The knowledge of the physicochemical properties of the micellar phases is required in order to use them properly. This is outlined in the following chapter. The use of surfactants in chromatography was first implemented in ion-pair chromatography. This led to the employment of

surfactants in thin layer chromatography and then in LC. This historical development is reviewed in Chapter 3.

An important property of surfactants is their ability to adsorb at any interface. Adsorption at stationary phases is discussed in Chapter 4, together with several related topics. Armstrong's early work proposed a retention model explaining the behavior of the solutes inside the column [3]. Later this model was slightly modified and adapted for very hydrophobic [19], micelle-repelled solutes [20] and hybrid micellar solutions containing organic modifiers [21]. Chapter 5 describes the partitioning theory, based on Armstrong's three phase model. These parameters are linked to thermodynamics. However, kinetics of all exchanges should also be considered because these are related to efficiency in chromatography. The reduced efficiency was a problem noted early in MLC. Solutions proposed to remedy these problems are described in Chapter 6.

True micellar phases (surfactant + water) can be considered mobile phases with low elution strength. If organic modifiers, mainly alcohols, are added in small proportions to the micellar phases, a significant enhancement of the efficiency as well as elution strength is observed. Chapter 7 is dedicated to this topic. When micellar solutions and/or hybrid micellar phases with alcohol additions are used as mobile phases, it is possible to accurately predict the retention of each compound in a mixture. This capability is more decisive than in conventional RPLC because the MLC peaks are broader. The elaborated equations for modeling and optimization purposes given in Chapter 8 are easily applied with the aid of the *MICHRON* software included with this book. Appendix I is given as an aid to run the software.

The octanol-water partition coefficient scale may not be the best tool for hydrophobicity evaluation. The ability of MLC for hydrophobicity measurement and some studies on quantitative structure retention activity relationships (QSAR) are described in Chapter 9. Chapters 10 and 11 contain selected examples of applications in the analysis of a variety of samples, especially pharmaceutical preparations and physiological fluids, some of them are taken from the authors own experience. Details on the nature of the sample, stationary phase, mobile phase composition, detection wavelength, and figures of merit, are tabulated at the end of each of these

chapters. Spectrophotometric detection has been utilized in most of the reported applications, but other detection modes are also interesting in MLC, as described in Chapter 12.

Chapter 13 provides other uses for organized media, such as microemulsions or supercritical fluids, in chromatographic separation. Micellar electrokinetic chromatography has attracted more attention than other separation techniques using micellar phases. The connection of this technique with MLC is also discussed.

Sodium dodecyl sulfate is the most commonly used surfactant in MLC. However, there are several thousand varieties of surfactants and many of them can be used as well. Appendix II tabulates the physico-chemical properties of selected surfactants. A collection of micellar partitioning coefficients gathered from the MLC literature are also given in Appendix III. Finally, Appendix IV shows examples of phase diagrams of a few systems relevant in MLC and the simplest way to make one that cannot be found in the literature.

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2

Physicochemical Properties of Micellar Media

I. INTRODUCTION

When the surfactant concentration is above a specific value: the *critical micelle concentration* (cmc), the surfactant solution becomes a micellar solution. Micellar liquid chromatography (MLC) uses solutions of surfactants forming micelles, as mobile phases. A basic knowledge on the organized surfactant systems is, thus, required to understand correctly the capabilities of this chromatographic technique. This is the purpose of this second chapter. The basic and simplified background knowledge on micellar and microemulsion systems is provided. The properties of surfactants, micelles and the parameters affecting the cmc value are exposed. The fundamental properties relevant to MLC are described extensively. The other properties are touched upon briefly.

For the convenience of the MLC user, it was deliberately chosen to refer to review articles or books rather than to the many original articles that establish the fundamentals of the physicochemistry of micellar media. For example, the Surfactant Science Series [1], edited by Schick and Fowkes (Marcel Dekker, Inc., NY) and the Surfactants in Solution collection, edited by Mittal (Plenum, NY) were extensively used to prepare this chapter.

II. SURFACTANT MOLECULES

The word *surfactant* is a contraction of surface active agent, referring to the main property of this class of molecules: their adsorption at any interface that modifies the surface or interfacial tension. Surfactant molecules are also called *detergents* and *amphiphiles* from the greek words "amphi" and

"philo," which mean respectively, both and loving. The amphiphile molecules love both polar and nonpolar media.

II.1. General Description

The amphiphilic character of surfactant molecules is due to the association of two parts with very differing polarities inside the same molecule [2]. One part is highly nonpolar, hydrophobic or lipophilic, usually an alkyl chain. Another part of the surfactant molecule is polar or hydrophilic. It can be a nonionic chain with polar groups, such as ether, alcohol or amine groups, or an ionic (anionic or cationic) group. Figure 2.1 shows the schematic representation of a surfactant molecule. Some surfactants have two nonpolar tails or two polar heads, as illustrated in the figure. The nature of the surfactant polar head is used to classify the molecules.

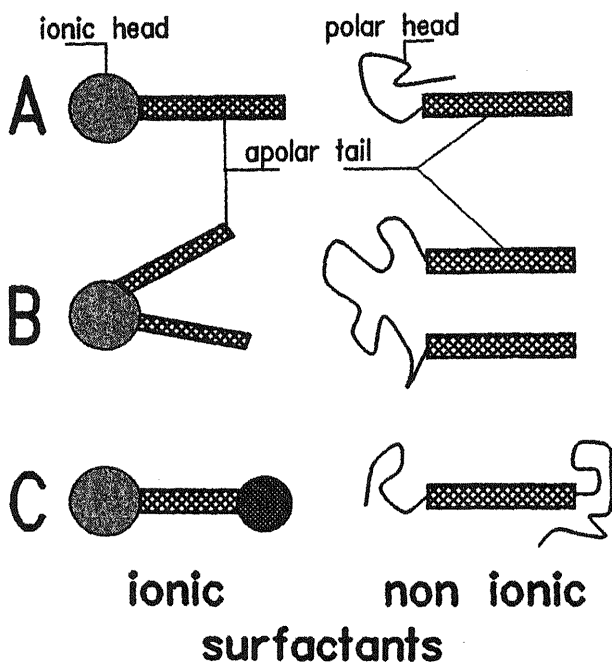


Figure 2.1 Schematic representation of ionic surfactants (left) and nonionic surfactants (right). A- classical surfactants (*e.g.*, SDS, CTAB, Brij); B- two-tailed surfactants (*e.g.*, AOT); C- two polar head surfactants (*e.g.*, betaine, Pluronic, Tween 80).

II.2. Classification

According to the electrical charge of their polar head, the three main classes of surfactants are: anionic surfactants, cationic surfactants, and nonionic surfactants. A fourth class is added for the amphoteric and/or multi-functional surfactants [3].

a) Anionic Surfactants

In aqueous solution, the anionic surfactant dissociates giving an anion carrying the amphiphilic properties and an inactive cation (*e.g.*, Na⁺ or K⁺). Anionic surfactants are the most commonly used active principles in industrial and household detergent preparations. Soaps, sulfonated compounds, alkylsulfates and alkylphosphates are the four main anionic surfactant families.

Table 2.1 Correspondence Between the Historical and Normalized Names of Fatty Acids Whose Salts Make the Soaps

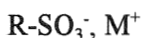
Carbon number	Historical name	IUPAC name
6	caproic acid	hexanoic acid
8	caprylic acid	octanoic acid
10	capric acid	decanoic acid
12	lauric acid	dodecanoic acid
14	myristic acid	tetradecanoic acid
16	palmitic (cetic) acid	hexadecanoic acid
16:1	palmitoleic acid	9-hexadecenoic acid
18	stearic acid	octadecanoic acid
18:1	oleic acid	9-octadecenoic acid
18:2	linoleic acid	9,12-octadecadienoic acid
18:3	linolenic acid	9,12,15-octadecatrienoic acid
20	arachidic acid	eicosanoic acid
20:4	arachidonic acid	5,8,11,14-eicosatetraenoic acid
22	behenic acid	docosanoic acid
22:1	erucic acid	13-docosenoic acid
24	lignoceric acid	tetracosanoic acid

Soaps. The saponification of natural oils and fats produces glycerol and soaps. The saponification of fats by fresh ashes is, probably, the oldest organic chemical reaction known. This is the reason why fatty acids are still named using ancient names differing from the IUPAC nomenclature. Table 2.1 lists the historical names along with the IUPAC names of the fatty acids, whose sodium or potassium salts are the constituents of soaps.

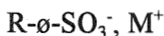
Soaps are most often a mixture of several sodium and/or potassium salts of fatty acids, present in the saponified natural oil. For example, the saponification of linseed oil by soda produces a soap whose composition is 51% sodium linolenate, 22% sodium oleate, 17% sodium linoleate, 6% sodium palmitate and 4% sodium stearate.

Soaps absorb weekly UV light at short wavelengths. The water solubility of fatty acids is very low and the protonation constant of the carboxylic acid group is $\log K \approx 5$, or $\text{pK}_A \approx 5$. The consequences of these two latter properties are: (i) soap solutions are moderately basic ($\text{pH} \approx 9$), and (ii) when the pH is decreased, the fatty acid corresponding to the soap in solution separates in a supernating, most often waxy, layer.

Sulfonated Compounds. These surfactants are the most important among the industrial surfactants [3]. The aqueous sulfonate solutions are neutral and very stable. In sulfonated surfactants, the sulfur atom of the hydroxysulfonyl group is directly connected to a carbon atom of the hydrophobic residue. The *alkylsulfonates* have the general formula:

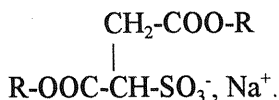


The alkyl chain, R, has 8 to 24 carbon atoms, and the metal cation, M^+ , is most often Na^+ , and sometimes NH_4^+ or K^+ . The alkylsulfonates do not absorb UV light at wavelengths longer than 200 nm. The *alkylbenzene-sulfonates*, with the general formula:



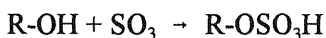
and 8 to 20 carbon atoms in the alkyl chain (σ means aromatic ring), are easy to produce industrially, being the most commonly used surfactants in detergent formulations and cleansing agents. The aromatic ring absorbs UV

light. The *sulfosuccinates* have two hydrophobic chains, and are salts of dialkylsulfosuccinate esters with the formula:



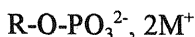
Aerosol® OT (sodium diethylhexyl sulfosuccinate) is a surfactant known to form readily reverse micelles, in organic nonpolar solvents [4]. Sulfosuccinates absorb slightly UV light at short wavelengths. However, the ester group is sensitive to hydrolysis, so they can only be used in near neutral solutions.

Alkylsulfates. The salts of sulfuric acid ester form the alkyl sulfate surfactant family. They are produced by sulfation with sulfur trioxide of linear alcohols:



The alkylsulfuric acid formed is a strong acid, easily neutralized by sodium or potassium hydroxide. The alkylsulfate aqueous solutions are neutral and stable in a wide pH range. Slight hydrolysis occurs in very acidic solutions. Sodium dodecyl sulfate (SDS), $\text{C}_{12}\text{H}_{25}\text{OSO}_3\text{Na}$ is the most common member of this family. Its physicochemical properties have been extensively investigated [3,4].

Alkylphosphates. This family includes the salts of the mono and dialkylesters of phosphoric acid.

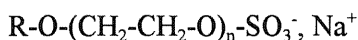


and

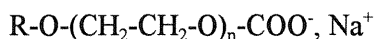


The alkylphosphates are low UV absorbing surfactants and are very stable in aqueous solutions, even at extreme pH values [4].

Other Anionic Surfactants. The hydrophilic part of many industrial surfactant molecules associates an anionic group with a nonionic one [3]. These surfactants have the general behavior of anionic surfactants. Two examples of such composite surfactants are the *alkylethersulfates*:

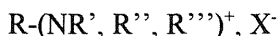


and the *carboxymethylethoxylates*:

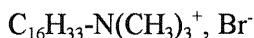


b) Cationic Surfactants

In aqueous solutions, cationic surfactants are ionized in a cation carrying the amphiphilic properties, and an inactive anion, such as Cl^- or Br^- . The cationic group is most often a quaternary ammonium group [5]. The general formula is:



with R, the hydrophobic chain. The R' , R'' and R''' groups can be identical or slightly different. The cetyl or hexadecyl trimethyl ammonium bromide (CTAB or HTAB):



is the cationic surfactant most studied. *Alkyl trimethylammonium salts* and *dialkyl dimethylammonium salts* are non UV-absorbing surfactants, very stable in aqueous solutions.

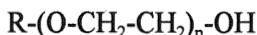
A variety of cationic surfactants are derived from pyridine and imidazole, such as the *alkyl pyridinium salts* and the *alkyl imidazolidinium salts*. These salts are very stable in aqueous solutions, but absorb UV light which makes them difficult to be used in MLC. Also, surfactants associating a nonionic polyoxyethylene chain with a cationic terminal group have been designed [5].

c) Nonionic Surfactants

Nonionic surfactants are not salts, obviously, they do not give ions in solution [6]. The hydrophilic part of their molecule contains polar groups

such as ether, alcohol, carbonyl or amino groups. Nonionic surfactants are stable in aqueous solution and are a little sensitive to the ionic strength and water hardness. A variety of nonionic surfactants are commercially available: 90% or more of these surfactants are obtained by polycondensation of ethylene oxide.

The *alkyl ethoxylates*:



can be referred to as C_mE_n , with m , the carbon number of the alkyl chain and n , the ethylene oxide number of the hydrophilic polyoxyethylene chain. These surfactants are non UV-absorbing molecules, useful in MLC. Due to the ethylene oxide polymerization step in their synthesis, the alkylethoxylates are mixtures of molecules with the same hydrophobic alkyl chain and a hydrophilic chain, having a different number of ethylene oxide units [6]. For example, the polyethylene 20 stearyl ether, marketed as Brij® 78, was found to contain only 30% of $\text{C}_{18}\text{E}_{20}$. It also contains 25% of $\text{C}_{18}\text{E}_{21}$, 25% of $\text{C}_{18}\text{E}_{19}$, 10% of a mixture of $\text{C}_{18}\text{E}_{22}$ and longer hydrophilic chains and 10% of a mixture of molecules with less than 19 ethylene oxide units.

It is possible to adjust both the hydrophobic chain length and the ethylene oxide condensation in the nonionic surfactant synthesis. The properties of the final molecule can be tuned well to the needs. Many chemical companies have developed a line of nonionic homologue surfactants. As an example, Table 2.2 lists the characteristics of the Brij® surfactants from the ICI Chemical Company [6]. The physicochemical properties of solubilization, emulsification, wetting, cleansing and detergency, foaming or antifoaming, are very different among the members of the Brij family. The hydrophilic-lipophilic balance value (HLB), defined as the ratio of the molecular weight of the hydrophilic group to the molecular weight of the nonionic surfactant time 20, is used to sort the surfactants in a 0-20 scale, from the more hydrophobic (low HLB value) to the more hydrophilic. Two surfactants with similar HLB values may have differing behavior, due to the very differing molecular weights. Furthermore, some batch to batch variation of the homologue distribution may occur for the same surfactant. It must be pointed out that the formula given in Table 2.2 correspond to the average homologue molecule. The actual distribution in

ethylene oxide units around the indicated value may vary widely. It can be noted that the CAS (Chemical Abstract Service) number given to the C_mE_n chemicals is often related to the alkyl chain length only.

Table 2.2 Alkyl Ethoxylate Nonionic Surfactants of the Brij® Series^a

Brij®	Formula	HLB	CAS number
30	$C_{12}E_4$	10.2	5274-68-0
35	$C_{12}E_{23}$	17.2	9002-92-0
52	$C_{16}E_2$	5.6	9004-95-9
56	$C_{16}E_{10}$	13.2	9004-95-9
58	$C_{16}E_{20}$	16.0	9004-95-9
72	$C_{18}E_2$	5.2	9005-00-9
76	$C_{18}E_{10}$	12.7	9005-00-9
78	$C_{18}E_{20}$	15.5	9005-00-9
92	$C_{18:1}E_2$	5.2	9004-98-2
97	$C_{18:1}E_{10}$	12.7	9004-98-2
99	$C_{18:1}E_{20}$	15.5	9004-98-2
700	$C_{18}E_{100}$	18.9	9005-00-9
721	$C_{18}E_{21}$	15.7	9005-00-9

^a C_mE_n is the alkylethoxylate surfactant with m ethylene oxide units and n carbon atoms in the alkyl chain. C_{18} and $C_{18:1}$ correspond to the stearyl and oleyl hydrophobic groups, respectively.

The *copolymers* are another family of non UV-absorbing nonionic surfactants, in which the hydrophilic chain is an ethylene oxide polymer, and the hydrophobic chain is a propylene oxide polymer. The two polymers can be connected in one or several points. For example, the Pluronic® series from the Wyandotte Chemicals Corporation are block copolymers with the general formula:

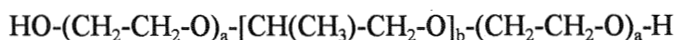


Table 2.3 Structure and Commercial Denomination of the Block Copolymer Nonionic Surfactants of the Pluronic® Series^a

<i>b</i>	Oxyethylene units (hydrophilic) in polymer (% w/w)						
	10%	20%	30%	40%	50%	70%	80%
16	L31 <i>1100</i>				L35 <i>1900</i>		F38 <i>4800</i>
21		L42 <i>1500</i>	L43 <i>1700</i>	L44 <i>2000</i>			
30	L61 <i>2000</i>	L62 <i>2200</i>	L63 <i>2500</i>	L64 <i>2900</i>	P65 <i>3500</i>		F68 <i>8700</i>
35		L72 <i>2800</i>			P75 <i>4100</i>	F77 <i>6800</i>	
39	L81 <i>2500</i>			P84 <i>3800</i>	P85 <i>4500</i>	F87 <i>7500</i>	F88 <i>11200</i>
47		L92 <i>3500</i>		P94 <i>4600</i>			F98 <i>14000</i>
56	L101 <i>3600</i>		P103 <i>4700</i>	P104 <i>5400</i>	P105 <i>6500</i>		F108 <i>16200</i>
69	L121 <i>4500</i>	L122 <i>5000</i>	P123 <i>5700</i>			F127 <i>13300</i>	

^a *b* is the number of oxypropylene units (hydrophobic) in the surfactant molecule.

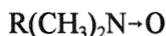
The room temperature appearance of the surfactant is: L = liquid, P = paste, F = flakes.

The numbers in italics under the codes are the surfactant average molecular weights.

By changing the oxyethylene (a) and oxypropylene (b) unit numbers, it is possible to build Pluronic homologues with a wide variety of properties. Table 2.3 lists the molecular weights of the various members of the Pluronic series. The manufacturer code includes a letter: L for liquid (low molecular weight), P for paste and F for flakes (solid with high molecular weight), and a two or three-digit number. The first or the first two digits correspond to approximately one fifth of the oxypropylene units in the molecule, and the last digit indicates the oxyethylene percentage in the molecule. For example, Pluronic F68 is a solid whose mean *b* value is about $6 \times 5 = 30$, and whose molecule contains 80% of polyoxyethylene. The approximate mass of the lipophile polyoxypropylene central block is 1740. This corresponds to 20%

of the molecular weight of F68, which is estimated to be $1740/0.20 = 8700$, with 7000 (80%) as the mass of the two hydrophilic polyoxyethylene blocks. The a value is close to 80.

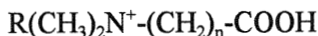
Other nonionic surfactant molecules include the *ethoxylated alkyl phenols*, which strongly absorb UV light, and the *ethoxylated fatty acids*, *fatty esters and alcanolamides*, which are slightly UV-absorbing nonionic surfactants [6]. The *amine oxides*, such as the alkyl dimethyl amine oxides:



are nonionic surfactants in basic and neutral solutions. They are protonated in acidic solutions and, thus, represent the transition to cationic surfactants.

d) Amphoteric and Multifunctional Surfactants

Amphoteric surfactants are ionic surfactants containing positive and negative charges on the same molecule [7]. They can be true amphoteric ions, such as the *betaines*:



with $n \geq 1$, that have a cationic nature in strongly acidic media and an amphoteric structure in neutral and basic solutions. These surfactants have a mobile proton with a pK_A value around 4. In *sulfobetaines*, the carboxylic group, $-COOH$, is replaced by a sulfonic group, $-SO_3^-$ ($pK_A < 1$). Sulfobetaines are amphoteric ions in the 1-14 pH range. Betaines with several methylene groups between the amino group and the acid group have been prepared. However, above three methylene groups, folding occurs since the positive charge is attracted by the negative charge. Such betaines have a nonionic surfactant behavior.

The *aminocarboxylic acids*:



are ampholytes: they are cationic surfactants in strongly acidic media, and anionic surfactants in basic media, and possess an isoelectric point at an intermediate pH value, with an inner salt amphoteric structure. Natural

L-amino-acids are the building blocks of proteins and made a science theme by themselves.

Other amphoteric surfactants include the betaines derived from imidazolines, which absorb UV light and the *lecithins* or phosphatidicholines, which are naturally occurring phospholipids [7]. Multifunctional surfactants associating a nonionic moiety with a charged one are considered ionic surfactants.

III. SURFACTANT SOLUTIONS

Surfactant molecules are water soluble and produce solutions with unique properties, due to the amphiphilic nature of the solubilized molecules. The two main properties of surfactant solutions are the *adsorption* at any interface and the *micelle formation*. These are a consequence of polar and nonpolar interactions with the surfactant molecules.

III.1. *Molecular Interactions and Surfactant Adsorption*

a) *Molecular Interactions*

The interactions between molecules can be sorted following an increasing polarity order as: (i) van der Waals forces, (ii) hydrogen bonding interactions, and (iii) electrostatic forces.

van der Waals Forces. These forces cover a variety of short distance range interactions, whose intensity decreases with the sixth power of the distance between two molecules. They include the *London* or dispersion forces between nonpolar molecules, the *Debye* forces between the permanent dipole of a molecule and the induced dipole of another molecule, and the *Keesom* forces or dipole-dipole interactions between two polar molecules. The order of magnitude of van der Waals forces is 2 kJ/mol for polar and moderately polar molecules, and less than 1 kJ/mol for nonpolar molecules [8].

Hydrogen Bonding. A hydrogen bond is the strong attraction between a hydrogen atom covalently bonded to an electronegative atom, and another electronegative atom. Only N, O and F are sufficiently electronegative to take part in hydrogen bonding in neutral molecules. The strength of a hydrogen bond is about tenfold higher than any van der Waals forces. It is about 20 kJ/mol for the $\text{O-H} \cdots \text{O}$ hydrogen bond [8].

Electrostatic Forces. Ion-ion interactions are long distance range forces, decreasing with the square of the distance between two ions. The ion-ion interaction strength is tenfold higher than the hydrogen bonding force, in the 200 kJ/mol range. Ion-dipole interactions have an intermediate strength. They are very important in the comprehension of the micelle formation of ionic surfactants.

b) *The Hydrophobic Interaction*

The notion of *hydrophobic interaction* was well developed by Tanford [9]. When a nonpolar solute is dissolved in water, some hydrogen bonds are disrupted. The solute tends to locally distort the water structure and to restrict the motion of water molecules. Thus, a large entropy increase in the water molecules is associated with the removal of the nonpolar solute from aqueous solution [9]. This entropy increase is responsible for the surface activity and micelle formation of surfactant molecules.

c) *Surfactant Adsorption*

The amphiphilic character of surfactant molecules explains their trend to adsorb at any interface. Two phases of different polarity are separated by an interface. This polarity difference attracts the surfactant molecules because this can minimize the entropy change by putting their polar part in the more polar phase and their nonpolar part in the less polar phase. Figure 2.2 shows the surfactant molecule arrangement at the liquid-liquid interface and at the liquid-air interface.

The adsorption of surfactant molecules at an interface decreases the interfacial tension. The decrease of the water-air interfacial tension explains the foaming property. The addition of a surfactant into a biphasic liquid system renders emulsion formation possible by the decrease of the liquid-liquid interfacial tension. Wetting and detergency are two important

properties of surfactant solutions due to surfactant adsorption at solid-liquid interfaces. The decrease of surface tension by surfactant adsorption at the water-air interface depends on the surfactant concentration. In MLC, the surfactant adsorbs on the stationary phase changing its nature and polarity.

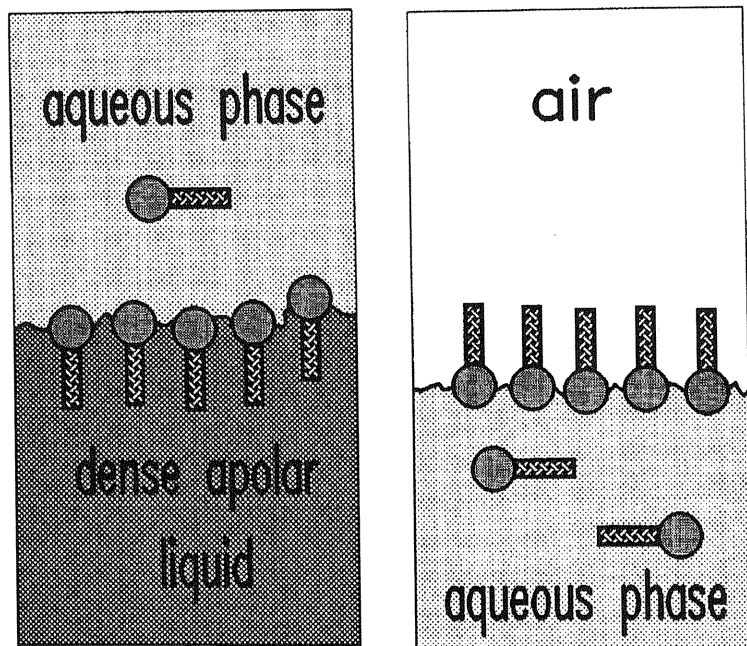


Figure 2.2 Surfactant adsorption at interfaces. Left, liquid-liquid interface, the hydrophobic tails points toward the less polar liquid phase. Right, air-water interface.

III.2. Micelle Formation

The second most important property of surfactant solutions is the micelle formation.

a) General Description

Figure 2.3 shows that the physicochemical properties of a surfactant solution, such as the surface tension, undergo an abrupt change over a narrow concentration range. This concentration is called the *critical micelle concentration* (cmc) of the surfactant. Above this concentration, micelles are present. Figure 2.4 shows a typical micelle for an ionic surfactant [10]. The surfactant molecules orientate in such a way that their polar heads face the water phase and their nonpolar tails aggregate in the waterless micelle core.

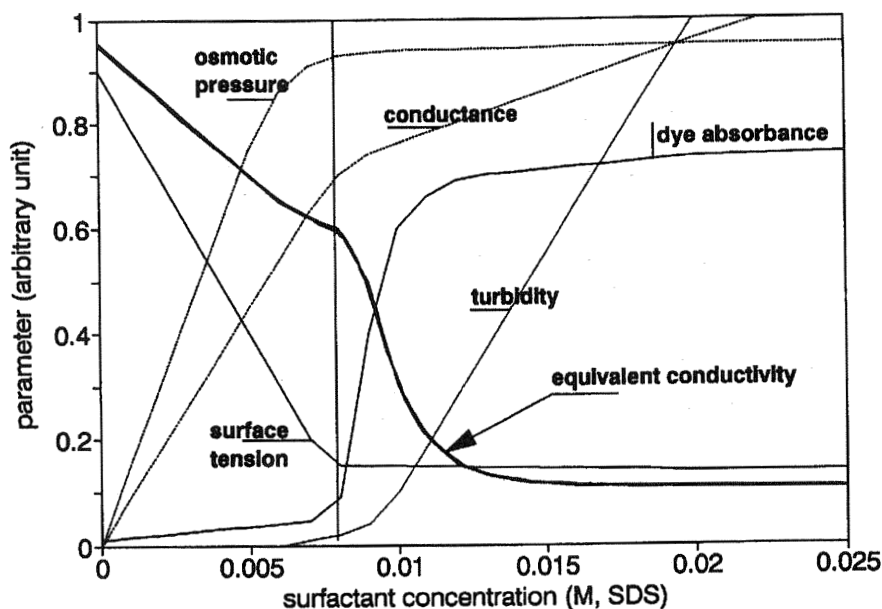


Figure 2.3 Physicochemical changes related to micelle formation at the critical micelle concentration (cmc). Density and refractive index are two parameters also affected by micellization (not shown).

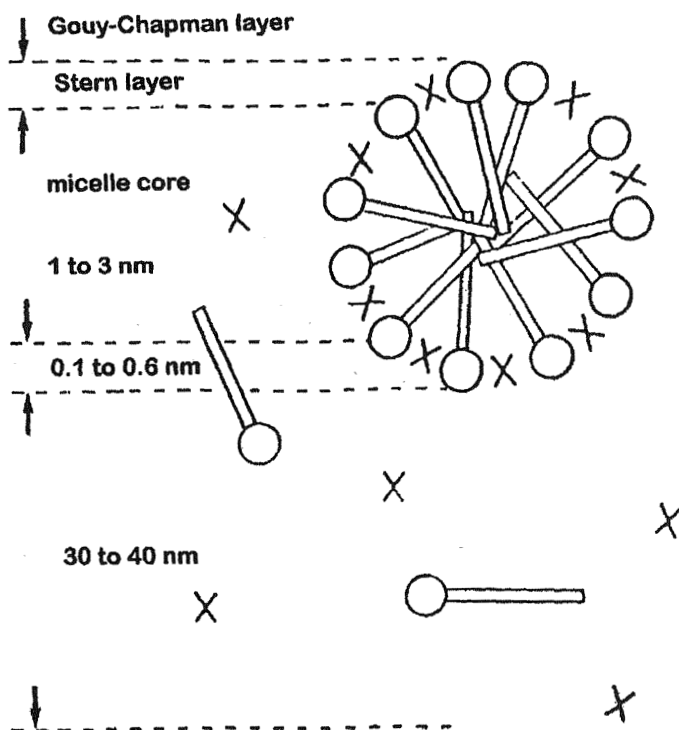


Figure 2.4 Representation of an ionic micelle according to Hartley [10]. The Gouy-Chapman layer corresponds to the diffuse layer under the electric field due to the micelle. The Stern layer corresponds to the ionic groups.

b) A Very Simple Model

Using a closed association model, micelle formation can be represented by:



in which N is the *aggregation number* or number of surfactant molecules, S , in the micelle aggregate. The equilibrium constant corresponding to Eq. 2.1 is:

$$K = [S_N] / [S]^N \quad (2.2)$$

Introducing the surfactant concentration, C , and the percentage of surfactant molecule forming micelles, Φ_m , the following is obtained:

$$K = \frac{C \Phi_m}{N[C(1 - \Phi_m)]^N} \quad (2.3)$$

Figure 2.5 shows the value of Φ_m for different sodium dodecyl sulfate concentrations. At a critical concentration, the cmc, the Φ_m increases sharply. The cmc can be calculated as:

$$\text{cmc} = (1 / NK)^{1/N-1} \quad (2.4)$$

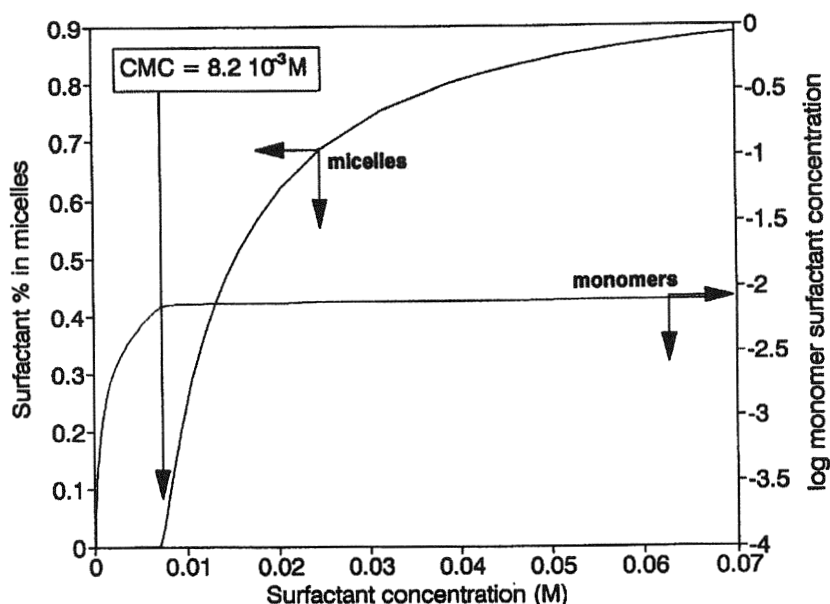


Figure 2.5 Micelle and monomer proportion related to the surfactant (SDS) concentration. Left ordinate: Amount of surfactant molecules in micelles (Φ in Eq. 2.3). Right ordinate: monomer surfactant concentration.

For SDS, the aggregation number is about 70 monomers of surfactant per micelle, and the cmc is 8.2×10^{-3} M at 25°C [4], which gives, using Eq. 2.4, the theoretical value $K = 10^{142} \text{ M}^{-69}$. The vast majority of surfactant molecules added above the cmc, self assemble in micelles [11]. The dotted line in Figure 2.5 shows the free monomer concentration, [S]. It is almost constant and equal to the cmc value at any concentration higher than the cmc. For example, the surfactant monomer concentration for SDS was calculated as 7.4×10^{-3} M, with a global surfactant concentration of 8.2×10^{-3} M (the cmc value). The micelle concentration was then only 1.1×10^{-5} M. With a tenfold increase in the overall surfactant concentration (8.2×10^{-2} M or 10 cmc), the micelle concentration was 1.06×10^{-3} M, two orders of magnitude higher than the value at the cmc, and the free monomer concentration was calculated as 7.9×10^{-3} M, only a 6% increase. Although very simple, the closed association model gives an approximation to surfactant micellization.

c) Thermodynamics of Micelle Formation

The free energy of micelle formation, ΔG° , is expressed by:

$$\Delta G^\circ = -RT/N \ln K \quad (2.5)$$

where R is the gas constant and T is the absolute temperature. Using the chemical potential, μ° , the law of mass action is:

$$K = \exp [N (\mu_s^\circ - \mu_M^\circ)/kT] \quad (2.6)$$

in which the subscripts S and M stand for surfactant monomer and surfactant in micelles, respectively.

From Eq. 2.4, it can be derived:

$$K \approx 1/N (1/\text{cmc})^{N-1} \quad (2.7)$$

combining Eqs. 2.5 and 2.7 and noting that the aggregation number, N, is in most cases higher than 20 gives:

$$\Delta G^\circ \approx RT \ln \text{cmc}. \quad (2.8)$$

Finally, the entropy change, ΔS° , due to the micelle formation is:

$$\Delta S^\circ = d\Delta G^\circ/dT = R \ln \text{cmc} + RT d(\ln \text{cmc})/dT \quad (2.9)$$

Increases in the cmc with temperature are often observed experimentally, which means that the ΔS° term is most often positive.

Micellar solutions are sometimes called *ordered media* [12]. The chemical order in a micellar solution seems to be greater than in a classical solution. Equation 2.9 shows that the micellization of surfactant molecules obeys the second principle of thermodynamics. It seems that the surfactant hydrocarbon chains have a much higher freedom of motion inside the micelle core than in the water bulk [13]. The micelle structure minimizes the molecule energy. The large entropy increase of water molecules associated with the removal of nonpolar surfactant tails from the aqueous solution (hydrophobic effect) is the main micelle driving force. Electrostatic forces tend to separate the polar heads that bear the same charge. The whole micelle is an equilibrium between these forces. This equilibrium is very sensitive to any chemical additive or parameter that can act on any of the forces, such as: salts, polar or nonpolar solutes, temperature and/or pressure.

III.3. The Micelle Nature

a) Micelle Fluctuations

The micelle structure is dynamic. The whole micelle aggregate has a finite mean lifetime. Furthermore, a given micelle existing at a given time, rapidly exchanges some surfactant monomers with the neighboring solution [14]. The dynamic character of micelles is characterized by two relaxation times, as illustrated by Figure 2.6 [15]. The first relaxation time, ranging from 10^{-8} s to 10^{-4} s, corresponds to a rapid monomer exchange between the micellar aggregate and the bulk solution. It can be considered as the mean lifetime of an individual surfactant molecule in the micelle [15]. The second relaxation time, in the range of 10^{-3} s to ~ 1 s, measures the lifetime of the aggregate itself. A decrease in size is first observed, followed by the vanishing of the aggregate, while other surfactant molecules are aggregating to form a new micelle (Figure 2.6).

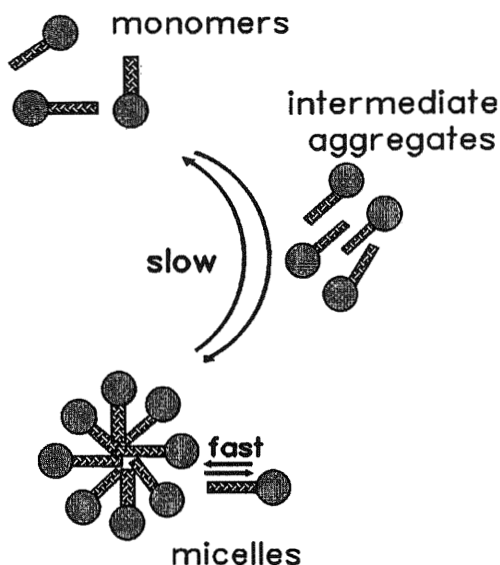


Figure 2.6 Dynamic exchange in micelles. Slow process (ms to s): micelle formation from monomers to micelle through intermediate aggregates. Rapid exchange (in the μ s range): exit and entrance of individual surfactant molecules.

b) The Micellar Pseudo-Phase

The micelle core is nonpolar. It contains some water molecules [16], but its overall polarity is much lower than that of water. The micelle core can be, thus, compared to a nonaqueous phase. However, due to the dynamic micelle fluctuations, it is not exactly a phase. The micellar phase is often called a *pseudo-phase*, because it is not possible to separate it from the aqueous phase. When the aqueous phase is removed by drying or evaporation, the crystalline form of the surfactant is obtained. This form is not identical to the micellar pseudo-phase, which is a hydrated form of surfactant molecules.

In a micellar solution, it is convenient to view the volume occupied by the surfactant molecules associated in micelles as a phase. From a

practical point of view, the volume percentage of the micellar phase, φ_m , is calculated as:

$$\varphi_m = (C - \text{cmc}) v \quad (2.10)$$

where v is the surfactant *molar volume* in L/mol, or volume occupied by one mole of surfactant in the aqueous solution. For example, in a 0.1 M SDS solution ($\text{cmc} = 8.2 \times 10^{-3}$ M, $v = 0.246$ L/mol), 2.26% of the volume is the micellar pseudo-phase, since $\varphi_m = 0.0226$ (Eq. 2.10). The aqueous phase occupies the remaining 97.74% of the liquid volume, corresponding to $1 - \varphi_m$. The φ_m value is used to obtain the aqueous and micellar pseudo-phase volumes, V_a and V_m , respectively:

$$V_a = V (1 - \varphi_m) \quad (2.11)$$

and

$$V_m = V \varphi_m \quad (2.12)$$

where V is the actual volume of the micellar solution.

These V_a and V_m volumes are used to evaluate the solute distribution between phases, the solute micellar *partition coefficient*, also called solute micellar *distribution constant*, and the solute local concentration in the micellar or aqueous phase.

c) Micelle Size and Shape

The micelle size depends on the aggregation number. However, the dynamic structure of micelles should be kept in mind. There is always polydispersity in micelle size due to the endless micelle creation/destruction process (Figure 2.6). The size distribution curve for the surfactant aggregates in 0.1 M SDS solution is illustrated in Figure 2.7. Both the so-called *monomers* and *micelles* are, in fact, an animated distribution of aggregates. The overall concentration of the SDS monomers is 8.0×10^{-3} M, roughly the cmc value of SDS (Figure 2.5), but only 3.5×10^{-3} M (44% of the non-micellized molecules) are true single monomers; 4.5×10^{-3} M (56%) are dimers, trimers with trace amounts of higher aggregates (Figure 2.7). The average SDS micelle aggregation number at 20°C and 0.1 M surfactant concentration is

62, although there is, actually, a Gaussian distribution centered on the value $N = 62$, with a variance of 13 [15].

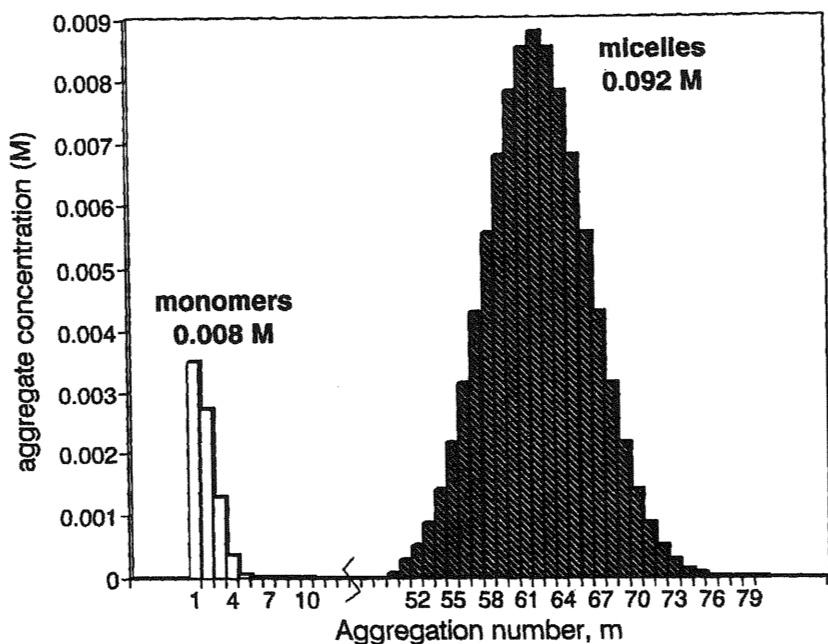


Figure 2.7 The distribution curve of the aggregates contained in a 0.1 M SDS solution at 20°C. The SDS micelle aggregation number varies from 50 to 80 with an average value of 62.

The micelle shape depends on the surfactant concentration. Spherical micelles (Figure 2.4) are found at a surfactant concentration near the cmc. The average diameter of SDS micelles is about 2 nm or $\sim 20\text{\AA}$ in concentrations near the cmc. As the surfactant concentration increases, the micelle size and shape can change. Oblong, rod-like or cylindrical micelles may be formed. The length and breadth of the rods or cylinders are in the low nm range (few tens of $\text{\AA}$). At high surfactant concentration, a viscous middle phase, a hexagonal structure and/or a neat phase with a liquid crystal structure are observed with some surfactants [17]. Figure 2.8 shows the molecular arrangement in some possible structures that may be found [18].

It is not possible to use viscous solutions as mobile phases in liquid chromatography. Only relatively diluted micellar solutions with spherical micelles will be useful.

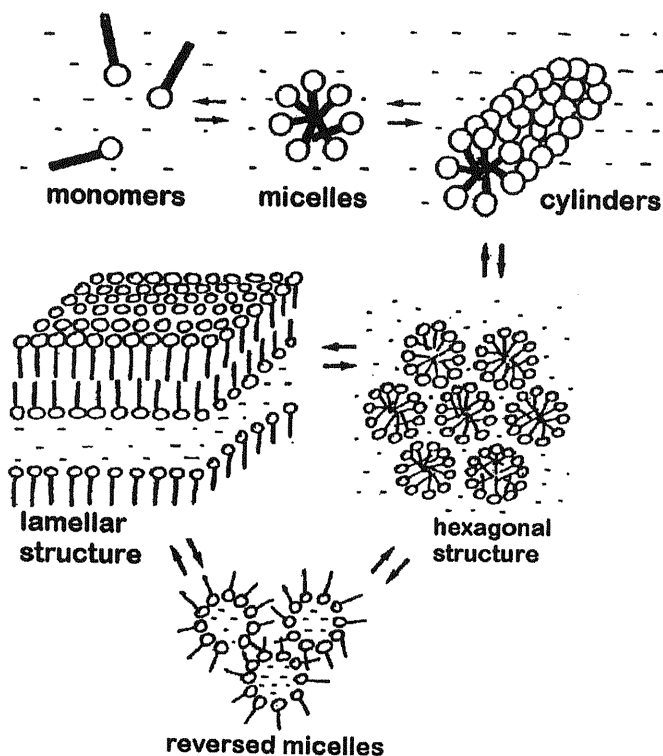


Figure 2.8 Possible physicochemical structures observed when the surfactant concentration in water increases. The lamellar and hexagonal structures have liquid crystal properties (from [18]).

III.4. Critical Micelle Concentration

As shown in Figure 2.3, the particular concentration at which numerous properties of the surfactant solution change, is the cmc of the surfactant. This value is very important to know. The micelle is formed by an equilibrium between the hydrophobic effect, due to the lipophilic part of the surfactant molecule, and the polar forces of the polar part of the molecule.

This equilibrium is sensitive to numerous parameters, and any change in the surfactant or experimental conditions, will affect the cmc value as described below.

a) *Surfactant Structure and the cmc*

The first parameter acting on the cmc value is the surfactant structure. A longer hydrophobic chain will render the surfactant more hydrophobic and promote micelle formation. For the homologous surfactant series, the cmc can be related to the alkyl chain length by:

$$\ln \text{cmc} = a - b n_c \quad (2.13)$$

in which a and b are constants, and n_c is the number of carbon atoms in the alkyl chain. Table 2.4 lists the regression parameters for Eq. 2.13, fitted with the cmc data obtained from members of the three main classes of surfactants. Table 2.5 gives the cmcs of the three homologous series. The polar head nature affects the cmc. The nonionic surfactants have much lower cmc values than ionic surfactants with similar lipophilic tail. The cmc value also decreases rapidly with the number of carbon atoms in the nonpolar alkyl chain. It can be seen that the cmc of the two ionic surfactant homologues decreases to a half when the nonpolar alkyl chain increases by one methylene unit. With one more methylene unit, the cmc of the nonionic surfactant homologues is decreased by a factor higher than 3. Therefore, the cmc of a non-ionic surfactant is two or three orders of magnitude lower than the cmc of the ionic surfactant with a similar alkyl tail.

Table 2.4 Regression Parameters of the $\ln(\text{cmc})$ vs. n_c Curves^a

Homologous series	a^a	b^a	$r^{2\ a}$
$C_nSO_4^- Na^+$	3.1 ^b	0.65 ^b	0.996
$C_nN(CH_3)_3^+ Br^-$	4.3	0.70	0.998
C_nE_3	4.8	1.21	0.999

^a a = intercept, b = slope, r = regression coefficient.

^b 40°C, other values are for 25°C.

Table 2.5 Experimental Values of cmc (mM) for Homologous Series of Surfactants

Carbon atoms	8	10	12	14	16	18
$C_nSO_4^- Na^+$	140	33	8.6	2.2	0.58	0.23
$C_nN(CH_3)_3^+ Br^-$	300	69	16	3.7	0.9	0.3
C_nE_3	7.8	0.69	0.058	0.006	$(5 \times 10^{-4})^a$	$(4 \times 10^{-5})^a$

C_n corresponds to an alkyl chain with n carbon atoms; C_nE_m is the alkylethoxylate surfactant with m ethylene oxide units and n carbon atoms in the alkyl chain. The cmc of the anionic alkyl sulfate surfactants are given at 40°C [19]. The cmc of the cationic [20] and nonionic surfactants [21] are given at 25°C.

^a Values estimated using the regression parameters of Eq. 13 given in Table 2.4 due to poor water solubility.

Table 2.6 Values of cmc (mM) for Different n -Dodecyl Surfactants at 25°C [22]

Polar head	$-O-SO_3^- Na^+$	$-SO_3^- Na^+$	$-SO_3^- \frac{1}{2}Ca^{2+}$	$-COO^- K^+$
cmc	8.2	10	1.3 ^a	12 ^b
Polar head	$-NH_3^+ Cl^-$	$-N(CH_3)_3^+ Cl^-$	$-N(CH_3)_3^+ Br^-$	$-CH(COO^-)_2 2 K^+$
cmc	15	20	15	130
Polar head	$-E_3$	$-E_{30}$	$-N(CH_3)_2^+ - (CH_2)_3-SO_3^-$ ^c	$-N(CH_3)_2^+ - COO^-$ ^d
cmc	0.058	0.080	1.2	1.5

^a At 54°C

^b Potassium tridecanoate or $C_{12}-COOK$

^c Dodecylsultaine

^d Dodecylbetaine.

Table 2.6 compares the cmc values of a number of *n*-dodecyl surfactants. It shows that the number of charges on the polar head is a parameter more important than the electrical sign (positive or negative). The counterion is equally important by its number of charges. Zwitterionic and, especially, nonionic surfactants have lower cmc values than the corresponding charged surfactants.

b) Effect of Temperature

The forces involved with the polar heads of ionic surfactants are electrostatic forces very different from the polar or hydrogen bonding forces involved with nonionic surfactants. Temperature acts very differently on these two types of forces, therefore, the effect of temperature on ionic surfactants should be studied separately from the effect on nonionic surfactants.

Ionic Surfactants. As early as 1895, Krafft [23] noticed that the solubility of ionic surfactants increased markedly above a particular temperature. Figure 2.9 shows the variation in solubility with temperature for SDS. Below 15°C, the SDS solubility is lower than 0.2% w/w. Above 15°C, the solubility increases dramatically: at 16°C, the saturated SDS solution contains 0.4% w/w of surfactant. At 19°C, the SDS concentration is higher than 4% w/w. The solubility increase is due to micelle formation. The *Krafft point* was described by Krafft himself as the melting point of the hydrated solid ionic surfactant in the aqueous phase. It was later found that this was coincidental. The Krafft point is now defined as the temperature at which micelles appear with the minimum surfactant concentration (Figure 2.9). Very often, the Krafft temperature or temperature at which a solution, at a given surfactant concentration (often 1% w/w) becomes monophasic, is given in the literature. The Krafft temperature is only a few degrees higher than the Krafft point (Figure 2.9). The *Krafft boundary* separates the micellar phase from the biphasic hydrated solid-liquid phase from the micellar phase or *L-phase*. The Krafft boundary terminates at high surfactant concentrations (30-40% w/w) when the L-phase changes into a hexagonal or lamellar phase. The Krafft point increases with the alkyl chain length of the surfactant. Also, it changes dramatically with the counterion nature, as shown in Table 2.7.

For ionic surfactants, the micelle structure is an equilibrium between the repulsion forces of the polar heads and the attraction forces between the

alkyl chains. The temperature may affect differently these two competing forces. The effect of temperature on the cmc values is often complex. At temperatures higher than the Krafft point, the cmc of most ionic surfactants increases slightly.

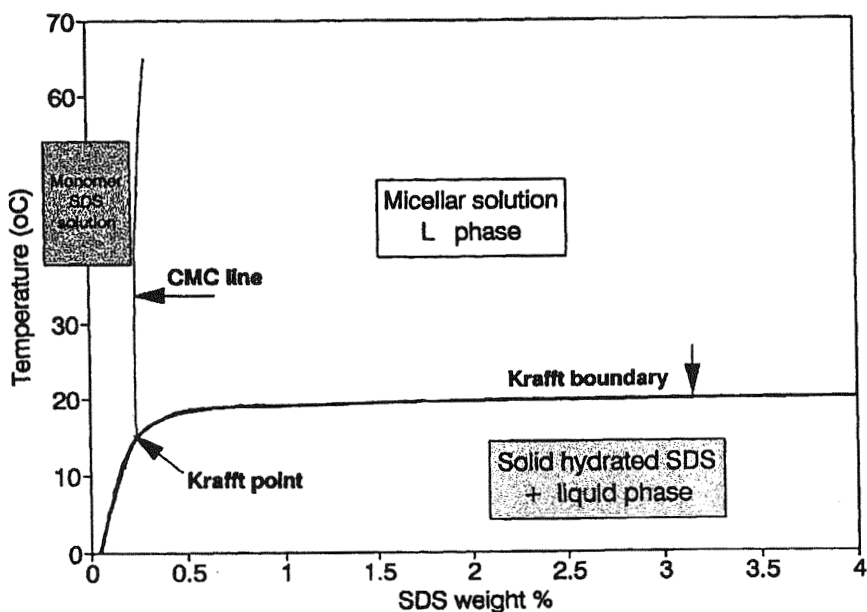


Figure 2.9 Temperature dependence of the SDS solubility. The Krafft point of SDS is close to 15°C. The Krafft boundary plateau is around 18°C.

A minimum cmc value can exist. This is the case of SDS, where the cmc is minimum at 29°C and increases at higher temperatures, as illustrated by Figure 2.10. A 20% increase in the cmc of SDS is noted between 30°C and 60°C. The cmc of dodecyl ammonium chloride, a cationic surfactant, is 1.5×10^{-3} M at 25°C (Table 2.6) and 1.7×10^{-3} M at 60°C. These examples give an idea of the magnitude of the effect of temperature on the cmc of ionic surfactants.

Table 2.7 Krafft Point ($^{\circ}\text{C}$) of Some Surfactants [13].

Polar head $-\text{O}-\text{SO}_3^-\text{Na}^+$	Alkyl chain			
	C_{10}	C_{12}	C_{14}	C_{16}
Krafft point	8	15	30	45
Surfactant	Sodium dodecanoate	Sodium tetradecanoate	Potassium tetradecanoate	Calcium tetradecanoate
Krafft point	36	53	8	$>100^a$

^a The high Krafft point of calcium surfactant salts is responsible for the precipitation of calcium soaps in hard waters.

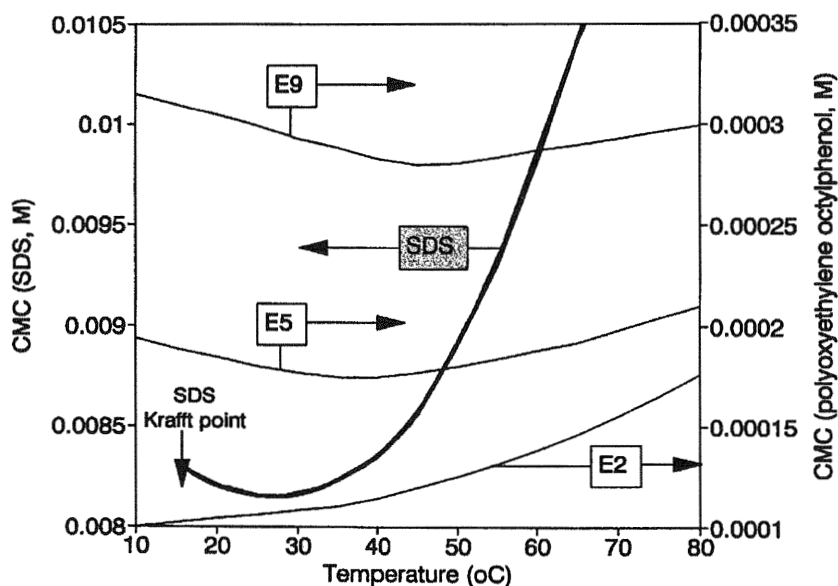


Figure 2.10 Temperature dependance of some surfactant cmcs. Thick line and left ordinate: SDS. Thin lines and right ordinate: polyoxyethylene octylphenol with two ethylene oxide units (E2), five units (E5) and nine units (E9).

Non-ionic Surfactants. These surfactants do not present the Krafft phenomenon. However, a nonionic micellar solution becomes turbid and separates in two phases when the temperature is raised. This is the *clouding phenomenon*. The polyoxyethylene chain, the polar part of most non-ionic surfactants, is progressively dehydrated as the temperature raises. Losing water molecules, the polyoxyethylene chain becomes less polar and, at a particular temperature, a turbidity, the clouding, appears. This temperature is called the *cloud point* of the nonionic surfactant solution. Above the cloud point, the nonionic micellar solution separates in an aqueous phase saturated by the nonionic surfactant, and an organic phase saturated by water and containing the major part of the surfactant.

Table 2.8 Cloud Point in °C of Some Pluronic® Nonionic Surfactants.^a

Surfactant Code	Ethylene oxide units ^b	1% Solution	10% Solution
L31	4	37	29
L61	6	24	17
L81	5.4	20	16
L101	8	15	11
L121	12	14	10
L35	22	77	80
P65	40	82	82
P75	46	82	86
P85	50	85	86
P105	74	91	90
F38	90	>100	>100
F68	160	>100	>100
F88	200	>100	>100
F108	300	>100	>100

^a Surfactants are described in Table 2.3.

^b In *Pluronic*® copolymers, the number of ethylene oxide units is referred as 2a, e.g. L31 contains 8 ethylene oxide units.

The cloud point temperature depends on the concentration of surfactant. It increases significantly with the polyoxyethylene chain length, but scarcely depends on the polar chain length. As an example, the cloud points of some nonionic Pluronic surfactants (see Table 2.3) are listed in Table 2.8. The nonionic micelle size and aggregation number increases dramatically with temperature. It can be stated that the aggregation number becomes infinite at the clouding temperature.

As illustrated by Figure 2.10 (polyoxyethylene octyl phenol surfactants with 2 (E2), 5 (E5) and 9 (E9) oxyethylene units), the cmc of nonionic surfactants may present a minimum value for a specific temperature. Often the increase of temperature produces a continuous increase of cmc (E2 on Figure 2.10). In all cases, the cmc variations with temperature are on the order of 1% per °C or less.

c) Effect of Added Electrolytes

The cmc of ionic surfactants is always depressed by the presence of added electrolytes. Shinoda [24] proposed the semi-empirical equation:

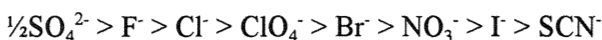
$$\ln (\text{cmc}) = -n_c \Delta G_m / kT - A\delta/z \ln (\text{cmc} + C_{aq}) + B \quad (2.14)$$

in which n_c is the carbon number of the surfactant alkyl chain, ΔG_m is the free energy difference per methylene group between the monomeric and micellar state, δ is the degree of counterion binding to the micelle, z is the valency of the counterion, C_{aq} is the added electrolyte concentration, and A and B are constants. This equation was proposed in a similar form by other authors [25, 26].

Eq. 2.14 shows that the cmc of ionic surfactants decreases exponentially with the concentration of any added electrolyte. It has been shown that the nature of the ion with the same charge as the ionic surfactant is not critical, *i.e.*, sodium chloride, sodium sulfate or sodium phosphate similarly decrease the cmc of sodium dodecyl sulfate [27]. However, potassium or cesium chloride would change the counterion binding value, α , which would produce a different slope in the $\ln (\text{cmc})$ versus $\ln (\text{cmc} + C_{aq})$ curve. For example, the cmc of SDS is 8.2×10^{-3} M at 25°C, is decreased to 3.1×10^{-3} M (a 62% decrease) by the addition of 0.03 M NaCl or 0.03 M

NaI, and to 2.1×10^{-3} M (a 75% decrease at 40°C), when 0.03 M CsCl is added [22].

The cmc of nonionic surfactants is also decreased by added salts [11]. The effectiveness of salts in altering the cmc of nonionic surfactants follows, approximately, the lyotropic series [11], which is for anions:



and for cations:



The magnitude of the effect is, however, much lower for nonionic than for ionic surfactants. For example, the cmc of $\text{C}_9\phi\text{E}_{15}$ is 1.1×10^{-4} M at 25°C. The addition of 0.03 M NaCl decreases the cmc by 5%, that is, only to 1.05×10^{-4} M. It starts to significantly decrease when the added salt concentration is above 0.1 M. Thus, to obtain a 55% decrease in the cmc, it is necessary to add as much as 0.86 M NaCl [22] (cmc = 5.5×10^{-5} M). When 0.86 M KNO_3 or NaSCN, or 0.43 M Na_2SO_4 or CaNO_3 are added, the cmc of $\text{C}_9\phi\text{E}_{15}$ becomes 5.5×10^{-5} M, 8.5×10^{-5} M, 2.3×10^{-5} M and 7.6×10^{-5} M, respectively [22].

The depression of the cmc by added salts is the result of a decrease in the repulsive forces between the ionic headgroups of the surfactant molecules. The micellization becomes easier, since the hydrophobic effect on the non-polar chains is little modified or slightly enhanced (salting out effect) by the added electrolytes [28]. Also, the aggregation number of ionic micelles enlarges dramatically which may change the micellar phase viscosity. The effect of salts on the cmc of a surfactant should be kept in mind when using MLC. It is often necessary to buffer the micellar phases using electrolytes.

d) Effect of Solvents and Pressure

The addition of an organic modifier is common in MLC, however, this can change the cmc value of the surfactant used. It is also important to consider the effect of pressure on the micellar state of surfactants, since pressure is a driving parameter in liquid chromatography.

Table 2.9 Effect of Short Chain Alcohols and of Pressure on the cmc of SDS and Decyl Trimethyl Ammonium Bromide (C₁₀TAB) at 25°C [43]^a

Alcohol				cmc (M)	
M	v/v %	w/w %	Mole fraction	SDS	C ₁₀ TAB
Methanol					
0.14	0.57	0.45	0.0025	0.008	0.063
0.88	3.6	2.8	0.016		0.059
1.9	7.7	6.2	0.035		0.063
4.0	16.2	13.2	0.079		0.068
5.9	23.9	20.0	0.122		0.082
Ethanol					
1.2	7.0	5.6	0.022	0.006	0.064
2.3	13.4	10.9	0.045		0.056
3.1	18	14.8	0.064		0.055
4.6	27	22.4	0.101	0.011	0.060
<i>n</i> -Propanol					
0.43	3.2	2.6	0.008	0.0038	0.051
0.94	7.0	5.7	0.018		0.039
1.5	11.2	9.2	0.030		0.031
2.3	17.2	14.3	0.048		0.019
Pressure				cmc (M)	
kg/cm ²		p.s.i.		SDS	C ₁₀ TAB
500		7000		0.0090	0.067
1000		14000		0.0094	0.067
2000		28500		0.0091	0.064

^a The cmc of SDS and C₁₀TAB in pure water are 0.0082 M and 0.068 M, respectively, at 25°C and atmospheric pressure.

Solvents. Short chain alcohols decrease the cmc of ionic surfactants at low concentration, but a cmc increase is also often observed for methanol and ethanol at larger concentrations (Table 2.9). Longer alcohols decrease the cmc, as illustrated by Figure 2.11. It has been observed that the longer the alkyl chain of the linear alcohol, the higher the effect on the cmc of potassium dodecanoate [29, 43]. Dioxane, the solvent with the lowest polarity and still fully miscible with water, decreases the solution average dielectric constant. It was generally found to increase somewhat the cmc of surfactants. For example, the cmc of SDS is 9.0×10^{-3} M (a 9% increase) and 3.0×10^{-2} M (+250%) in a 10% w/w and 25% w/w dioxane solution, respectively, at 25°C [22]. Urea is a compound known for modifying the liquid water structure and for increasing the solution dielectric constant [11]. It also slightly increases the cmc of ionic surfactants. For example, the cmc of SDS is unaffected by 2 M urea and becomes 9.0×10^{-3} M (+9%) in a 3 M urea solution at 25°C [22]. The cmc of dodecyl trimethylammonium bromide is 1.5×10^{-2} M in pure water, it becomes 2.0×10^{-2} M (+33%) and 4.5×10^{-2} M (+200%), in 2 M and 6 M urea solutions, respectively [22].

Pressure. Table 2.9 also lists the effect of pressure on the cmc of SDS and CTAB. Extreme pressures (2000 kg/cm² or 28,500 p.s.i.) produce only small changes (<10%) in the cmcs. It can be considered that the pressure used in classical liquid chromatography, in the 40-200 kg/cm² range, will not change the cmc of the surfactant used.

e) Determination of cmc Values

Figure 2.3 shows a variety of physicochemical changes of the surfactant solution occurring when micelles form. The measurement of any one of these properties can be used to determine accurately the cmc of a surfactant.

Surface Tension Measurement. The most commonly used cmc determination method is the surface tension measurement [22]. Figure 2.2 shows that the surfactant molecules orientate at the solution air-water interface. This surfactant adsorption decreases the surface tension. The magnitude of the tension decrease depends on the free monomer surfactant concentration. As shown by Figure 2.5, the free surfactant monomer concentration reaches a plateau for surfactant concentrations above the cmc. As the surfactant concentration is increased in the aqueous solution, the surface tension

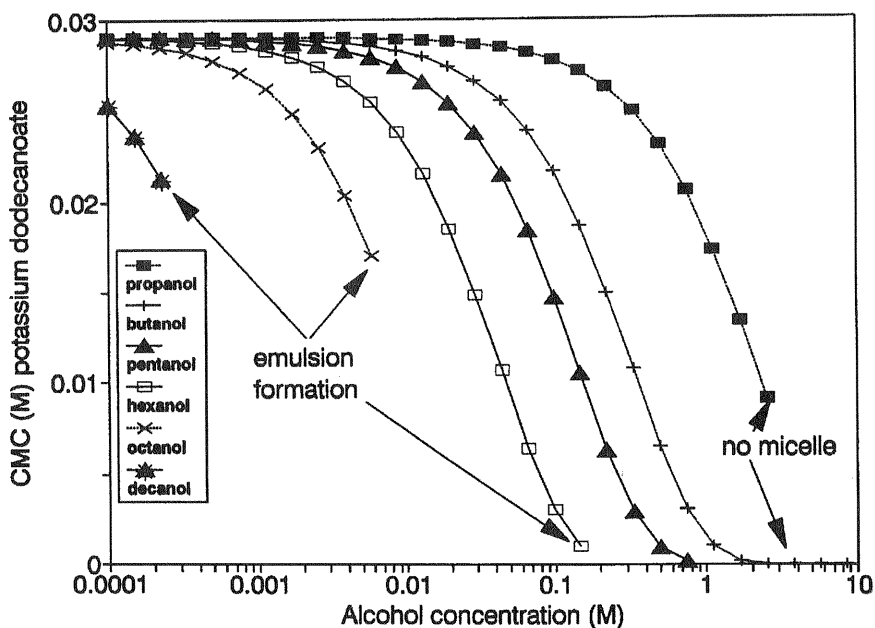


Figure 2.11 Effect of normal alcohols on the CMC of potassium dodecanoate (25°C). Micelles does not form in concentrated alcohol solution (3M propanol = 23% v/v; 3M butanol = 27.5% v/v). Milky emulsion formation occurs with very low amounts of long chain alcohols (from [29]).

decreases from 72.75 dynes/cm (0.07275 N/m), the surface tension of pure water at 20°C, to few dynes/cm (10^{-3} N/m) when the cmc is reached and exceeded. The extrapolation of the decreasing part of the surface tension vs. log (surfactant concentration) plot (below the cmc) and the horizontal part of the plot (above the cmc) gives the cmc value. Surface tensions are measured using a Du Nouy ring tensiometer, a Wilhelmy plate balance or apparatuses measuring drop weight, shape or volume. Surface tension measurement can be done with any surfactant.

Conductivity Measurement. The equivalent conductivity of micelles is lower than the one of free ionic surfactant monomers. Surfactant micellization produces a break in the conductivity vs. surfactant concentration curve. Both the specific conductivity or the equivalent

conductivity can be used for cmc determination [22]. Conductivity measurement is an accurate way to determine ionic surfactant cmcs in aqueous solutions. It should be used in organic rich solutions (*e.g.* 50% v/v methanol solution) to check if micellization does occur with ionic surfactants.

Spectrophotometry. The absorption spectrum of numerous dyes changes when they solubilize in the micelle core. Other dyes are essentially water-insoluble, they dissolve significantly in the presence of few micelles. The absorption spectrum of dye-containing surfactant solutions is recorded for increasing surfactant concentrations. When micellization occurs, an abrupt spectrum change is easily detectable. Sometimes a color change can be determined visually. Quinaldine blue (pinacyanol chloride), fluorescein, rhodamine 6G and bromophenol blue are examples of dyes whose spectrum changes upon micelle solubilization. Orange OT (1-*o*-tolylazo-2-naphthol), scarlet red (Sudan IV) or methyl yellow 2 (*p*-dimethylaminoazobenzene) are water-insoluble dyes used for cmc determination [22].

Other Methods. Turbidimetry, light scattering and refractive index measurements are spectrophotometric methods used to obtain surfactant cmcs. Viscosity, diffusion coefficient measurements, flow injection analysis and electrochemical methods (potentiometry and polarography and even capillary electrophoresis) can also be used.

The accuracy of the quoted methods is variable. However, all of them can rapidly give an essential information: a break in the studied property is a reasonable proof for micelles formation. A continuous change of the studied property should be a warning signal: is there any micelle in this medium?

IV. SOLUBILIZATION IN MICELLAR PHASES AND MICROEMULSIONS

A big interest in micellar solutions is that these media are able to solubilize, at the same time, polar, ionic solutes and nonpolar solutes. The location of the solubilized compounds in the micelle or surroundings depends on the polarity of the solutes. On the other hand, when the amount of nonpolar solvent incorporated in a micellar phase becomes high, emulsion and

microemulsion structures can be formed. Classical emulsions cannot be used as mobile phases in liquid chromatography, but microemulsions can. The microemulsion physicochemical structure and properties are exposed briefly below.

IV.1. Solubilization in Micellar Media

a) Solubilization Sites

Small amounts of nonpolar compounds can dissolve in the nonpolar core of the micelle, ionic compounds are located in bulk water, and polar compounds can partition between the polar layer (Stern layer in ionic micelles, Figure 2.4) of the micelle and bulk water. The polar layer of the micelles formed by nonionic surfactants is larger than the Stern layer of the ionic micelles (Figure 2.12). In nonpolar solvents, water and polar solutes are located in the core of reverse micelles, and nonpolar solutes are dissolved in the nonpolar solvent (Figure 2.8).

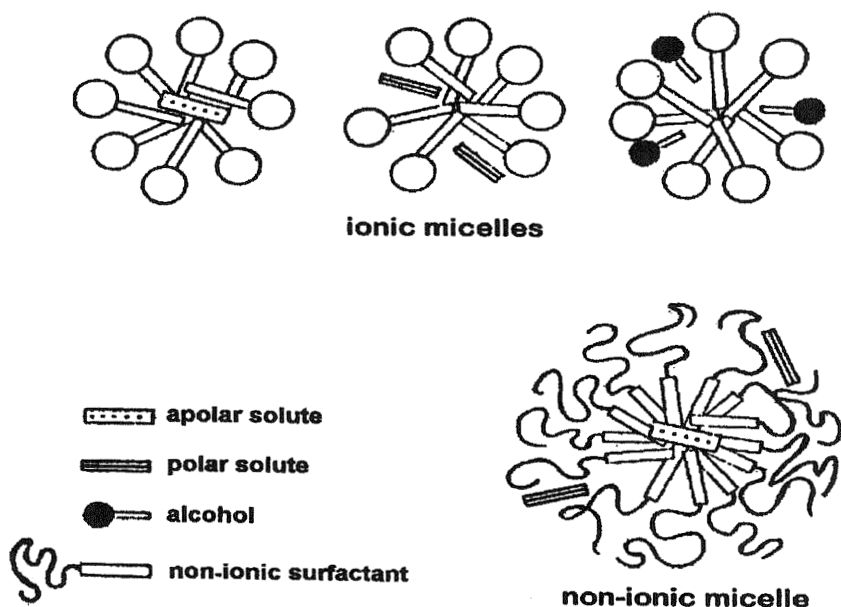


Figure 2.12 Localization of solutes in the micelles. The apolar solutes are located in the micelle core. The polar solutes are located in the ionic palisade (Stern) layer. Alcohols may form mixed micelles.

Often, the solubilized compound modifies the micellization process. As described above, the cmc can be increased or decreased. The solubilization of large amounts of nonpolar solutes can produce swollen micelles that is the first step towards emulsion formation. Butanol and longer alcohols tend to solubilize in micelles as illustrated in Figure 2.12. The polar hydroxyl group of the alcohol is located in the polar micelle layer, the alkyl chain is located in the nonpolar micelle core. These alcohols decrease the electrical repulsion between the charged heads of ionic surfactants. This is the first step towards microemulsion formation. What is a *mixed micelle*? This term means that the micelle has solubilized a significant amount of medium or long chain alcohol. There is no clear limit. If the calculation shows that there is 1 alcohol molecule every 4 or 5 micelles, it is not possible to speak of mixed micelles. In a mixed micelle, the number of alcohol molecules should be comparable to the number of surfactant molecules. A mixed micelle should contain several alcohol molecules per micelle.

b) The Micellar Partition Coefficient

When a solute is added to a micellar solution, it distributes itself between the aqueous solvent and the micelles. To quantitate this distribution, the *micellar partition coefficient* or *micellar distribution constant*, K_{MW} , is defined as the ratio of the solute concentration in the micelles to the solute concentration in the nonmicellar phase. It is expressed by [30]:

$$K_{WM} = \frac{N\rho[S]/\varphi_m}{(1-\rho)[S]/(1-\varphi_m)} \quad (2.15)$$

where N is the aggregation number, $[S]$ the solute concentration in the micellar medium, ρ is the mole percentage of the solute in the micelles, $(1-\rho)$ is the solute fraction located in the aqueous pseudo-phase, and φ_m the volume percentage of the micellar pseudo-phase (Eq. 2.10). The aggregation number is not always known, therefore, the solute partition coefficient per surfactant molecule, P_{WM} , is often used in MLC:

$$P_{WM} = \frac{K_{WM}}{N} = \frac{\rho(1 - \phi_m)}{(1 - \rho)\phi_m} \quad (2.16)$$

The solute partition coefficient and its location are linked. Figure 2.13 shows the plot of ρ values vs. surfactant concentration in SDS solutions. Six solutes with K_{WM} increasing from 100 to 200,000 are represented. Only 4% of the molecules of a solute 100-fold more soluble in the micelles than in water ($K_{WM} = 100$) are located in the micellar phase of a 0.12 M SDS solution. The reason is that the micellar phase volume is only 2.7% of the total volume. Otherwise, 42% and more than 99% of the molecules of a solute with K_{WM} values of 2000 and 200,000, respectively, are located in the micellar phase of the 0.12 M SDS solution. This shows that a solute with a $K_{WM} = 200,000$ can be considered fully located in the

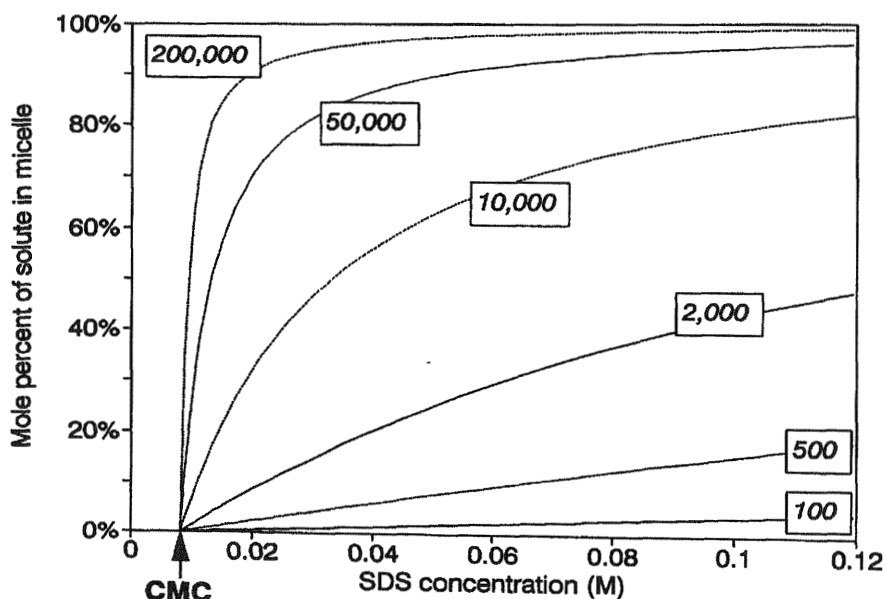


Figure 2.13 Localization of solutes in the micellar phase as a function of the solute partition coefficient. The indicated value is the solute partition coefficient, K_{WM} . It should be divided by 62 (SDS aggregation number) to obtain the distribution constant per SDS molecule, P_{WM} .

0.12 M SDS micellar phase. Solutes with K_{WM} values between 1000 and 20,000 are located in both phases. Solutes with lower K_{WM} values are located mainly in the aqueous pseudo-phase of the 0.12 M SDS solution. This SDS concentration is commonly used in the micellar mobile phase used in MLC. If the analyzed solute interacts with the SDS micelles, it means that its K_{WM} constant is higher than 1000. The aggregation number of SDS is about 62 molecules per micelle for 0.12 M SDS. Then, it should be noted that a value $K_{WM} = 1000$ corresponds to a distribution constant per SDS molecule of only $P_{WM} = 16$.

c) Solubilization of Salts and pH

Salts and ionic compounds have a high affinity for the aqueous pseudo-phase. Their distribution constant is, thus, close to zero. This means that they are completely excluded from the micelles. The consequence is that the concentration of salts in aqueous solvent may be higher than the bulk concentration. For example, for 1 M SDS (288 g/L), the volume percentage of the micellar pseudo-phase is $\varphi_m = 0.246$ (Eq. 2.10). Therefore, one liter of 1 M SDS solution contains 246 mL of micelles and 754 mL of water. If 0.01 M of a salt, say sodium nitrate, is dissolved in this micellar solution, the ions are located in the aqueous pseudo-phase, where its concentration can actually be 0.0133 M, 33% higher. The actual ion concentration will be $1/(1 - \varphi_m)$ higher than the overall concentration.

Ion location is even more important when considering the pH of a micellar solution. The proton concentration in the aqueous pseudo-phase will be:

$$[H^+]_a = [H^+]/(1 - \varphi_m) \quad (2.17)$$

where $[H^+]$ is the concentration of protons introduced in the micellar media. A similar equation can be written for the hydroxide ions. The autoprotolysis constant, K_S , or ionic product of water in the micellar medium can be written as:

$$K_S = [H^+][OH^-] = [H^+]_a[OH^-]_a(1 - \varphi_m)^2 \quad (2.18)$$

The pK_S value of a micellar solution will be expressed by [31]:

$$pK_s = -\log K_s = 14 - J - 2 \log (1 - \varphi_m) \quad (2.19)$$

at 20°C. The J value was introduced to take into account ion-exchange phenomena or electrical interactions with the organic interphase that may act on the ion location [31].

To illustrate the use of Eqs. 2.17-2.19, let's suppose that 0.01 M HCl is added into one liter of the 1 M SDS solution previously mentioned. It should be pointed out that a 1 M SDS solution is a highly concentrated micellar solution. The aqueous pseudo-phase pH is 1.87 and not 2.0, the pH of a classical solution; the pK_s value in 1 M SDS is 14.25, slightly higher than the value in pure water (14.00). The pH shift is only 0.13 unit (6.5%) with a φ_m value of 24.6%. The pH shift is smaller than 0.05 in micellar media with φ_m values lower than 10%, such as those used as mobile phases in MLC. This pH change can, consequently, be ignored. However, when concentrated emulsions or microemulsions are used, the pH shifts must be taken into account.

IV.2. Microemulsions

Micellar solutions are two component mixtures: water and surfactant. When a small amount of a third component is added, it can be solubilized in the micellar media. When a large amount of a third nonpolar component, called "oil," is added to a micellar solution, the solubility limit is exceeded and another system, biphasic in nature, forms an emulsion.

a) Emulsions and Microemulsions

The emulsion structure is obtained by adding energy to the mixture of water, surfactant and oil. The energy is necessary to increase the interface area. Droplets tend to form. Oil in water (O/W) emulsions are formed when the oil droplets are dispersed in a water continuous phase. Water in oil (W/O) emulsions have a continuous oil phase with water droplets inside (Figure 2.14). The droplet diameter range from 0.02 μm for very fine emulsion to 50 μm and more for coarse emulsions. The emulsion structure is not stable. With time, the droplets fuse by coalescence. Creaming or sedimentation

occurs and, eventually, the oil phase separates from the aqueous phase [32]. The instability and turbidity of such systems proscribe their use as mobile phases in MLC.

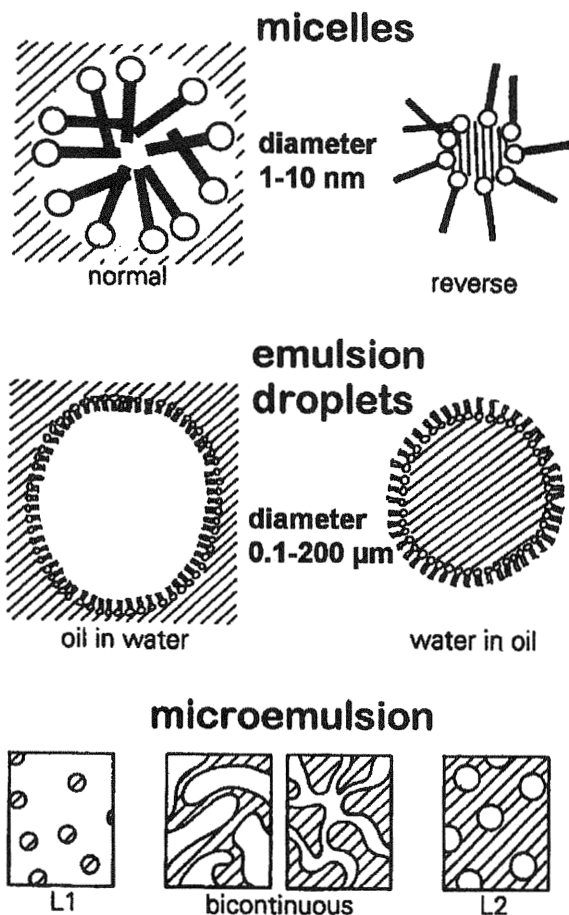


Figure 2.14 Physicochemical structures of the organized liquid systems. Micellar solutions, emulsions and microemulsions. Open areas: aqueous phase; dashed areas: apolar phase, redrawn from Ref. [31].

A microemulsion is an emulsion with droplets so small (<40 nm) that they do not diffuse visible light (Figure 2.14). A microemulsion looks

like a clear and transparent liquid phase with a low viscosity. It can, therefore, be used as a mobile phase in MLC. Most often, a fourth component is added to the water/surfactant/oil mixture to obtain the formation of O/W or W/O microemulsions, with just a gentle mixing [33]. The fourth component is called the *cosurfactant*, because it has some properties related to surfactants. It is, generally, a linear medium chain alcohol such as *n*-butanol, *n*-pentanol or *n*-hexanol. The alcohol and the surfactant associate together to form the *interphase*, the layer that separates the aqueous phase from the oil phase without very evident interface. Such surfactant-alcohol association produces a very low interfacial tension. It can be so low that the interfacial area tends to increase spontaneously [34].

Some microemulsions are thermodynamically stable, *i.e.*, they form immediately, and the oil and water phases cannot be separated without an energy input. However, most systems are not thermodynamically stable, although they always present a long term kinetic stability. If the water and oil phase separation does occur in a microemulsion system, the process will take several orders of magnitude longer (months to years) compared to the phase separation time interval of a classical emulsion [33,34].

b) Phase Diagrams

Any mixture of three components, A, B and C, can be represented using the properties of an equilateral triangle (ternary diagrams). A parameter: volume, mass or mole, is selected. The relative amounts of A, B and C are expressed in percentage of the selected parameter, such as:

$$A\% + B\% + C\% = 100\% \quad (2.20)$$

Only two percentages are independent, the third one can be obtained from Eq. 2.20. Figure 2.15 shows how a given mixture can be plotted. Point M is obtained at the crossing of the respective percentage values of A, B and C. Its composition is A: 59%, B: 9% and C: 32%. The apex of the triangle corresponds to the pure component. Water or the polar solvent is usually located at the lower left corner of the triangle. The lower right apex represents the oil or the non-polar solvent, and the top apex is the surfactant.

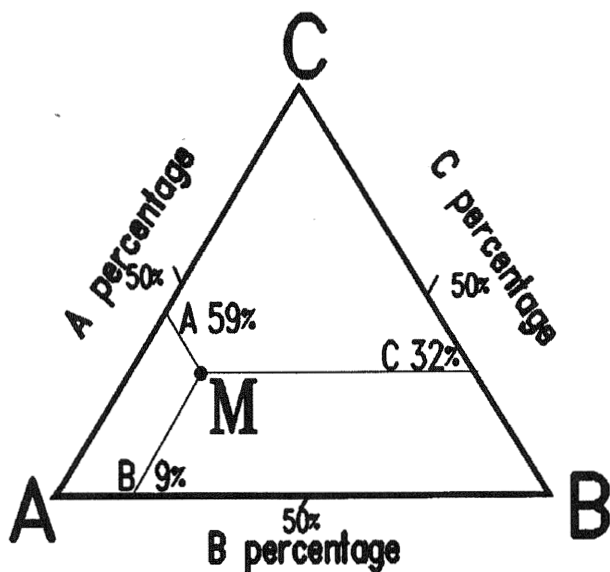


Figure 2.15 The ternary phase diagram representation. Point M represents the liquid composition with 59% of A, 9% of B and 32% of C (total 100%). The percentage can be mass, volume or mole percentages.

Most microemulsions are made with four components. In this case, Eq. 2.20 cannot be used, unless a pseudo-component is defined, such as a given ratio of surfactant to alcoholic cosurfactant. This active mixture is considered as the third component and is placed at the C apex. Figure 2.16 shows the phase diagram of the ternary system water/heptane/sodium bis (2-ethylhexyl) sulfosuccinate (Aerosol OT or AOT) [35]. AOT is an anionic surfactant able to form W/O microemulsions without the need of a cosurfactant. Figure 2.17 shows the phase diagram of the pseudo-ternary system water/heptane/(CTAB + *n*-butanol) [31]. CTAB is a cationic surfactant that needs to be associated with a cosurfactant to form microemulsions. The ratio CTAB/butanol was constant (1/1 w/w) for all compositions represented in the phase diagram. The hatched areas corres-

pond to emulsions or polyphasic mixtures. The open areas show the micro-emulsion domains and the dotted areas correspond to liquid crystal domains.

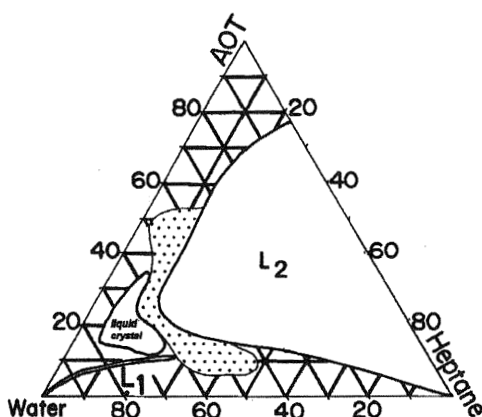


Figure 2.16 Mass phase diagram of the ternary liquid system made of sodium diethylhexyl sulfosuccinate (AOT), heptane and water (25°C). This system presents a vast L₂ (see Figure 2.14) area. The L₁ area is lenticular. The dotted area is a viscous, birefringent and milky area, probably a suspension of liquid crystal in L₂ phase, redrawn from [36].

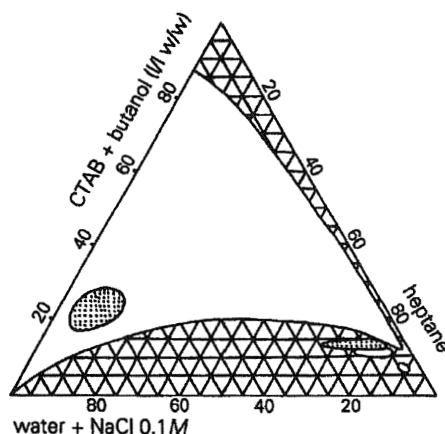


Figure 2.17 Mass phase diagram of the quaternary liquid system made of CTAB/butanol (1/1 in mass) as the surface active mixture (surfactant + cosurfactant), heptane and water (NaCl 0.1M). This system presents a continuous change from the L₁ structure to the L₂ structure (see Figure 2.14). The dotted area corresponds to viscous milky compositions, redrawn from [31].

c) *The Microemulsion Structure*

Phase diagrams are used to study the microemulsion structures. For example, Figure 2.18 shows nine phase diagrams that were used to study the structure of water-SDS microemulsions, obtained with different alcohols and oils [36]. The study of the microemulsion structure showed that three main structures were observed: (i) the O/W microemulsion structure, also called L1 structure, with microdroplets of organic phase in a continuous aqueous phase, (ii) the W/O structure, also called L2 structure, with microdroplets of aqueous phase in a continuous organic phase, and (iii) an intermediate sponge-like structure, called the *bicontinuous* structure [37, 38]. The microdroplets size of the structured L1 and L2 forms is in the 5-50 nm range (Figure 2.14).

The larger realms of microemulsion compositions were obtained with butanol as the cosurfactant. A continuous change between the L1, the bicontinuous, and the L2 structures, was observed as the amount of oil was increased in the system composition. With pentanol, the W/O microemulsion domain, also called L1 microemulsions, is separated from the O/W (or L2) microemulsion domain. With hexanol as the cosurfactant, the W/O or L1 microemulsion is composed of swollen micelles with more than 99% of water, and only the L2 structure is observed with the three oils studied (Figure 2.18) [36]. Many microemulsion compositions have a viscosity higher than 5 cP, which is the maximum value for the viscosity of a mobile phase in liquid chromatography [35].

Nonpolar solutes are located in the nonpolar organic phase, and polar solutes are found in the aqueous phase of the microemulsion. It should be noted that the interphase surfactant/cosurfactant can represent a significant part of the total mass or volume of the microemulsion. The ϕ_m value in a microemulsion system includes the oil, the surfactant and the cosurfactant, thus, ϕ_m values as high as 90% are possible in these systems. Solutes with intermediate polarity can partition between the two phases and/or be located in the interphase. Solute location in physicochemical structures stabilized by surfactants is the subject of continuous research [39-42].

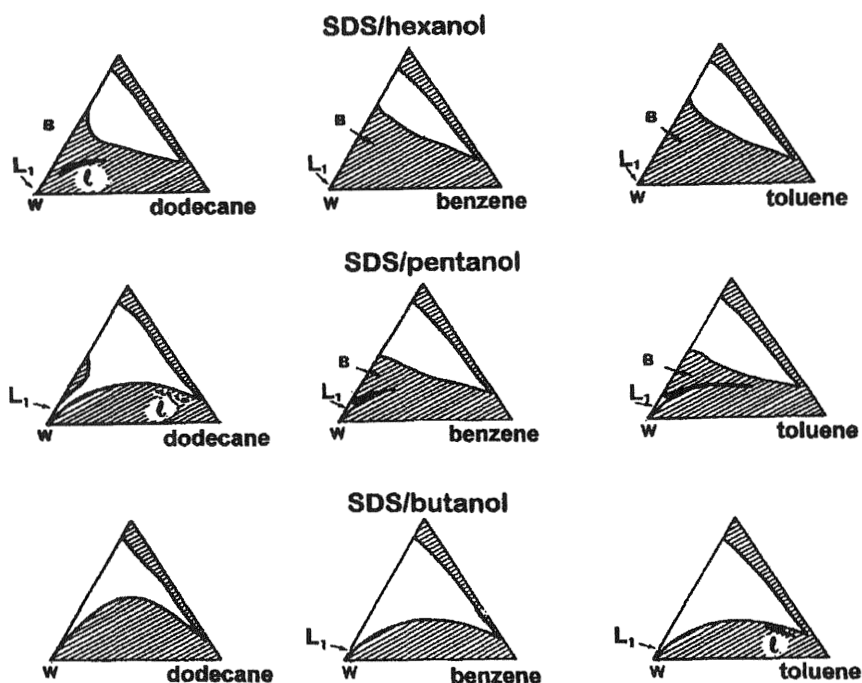


Figure 2.18 Evolution of the microemulsion realm of existence according to oil variation (from left to right: dodecane, benzene or toluene) and alcohol cosurfactant variation (from top to bottom: hexanol, pentanol, butanol, to the same 2/13 SDS/alcohol molar ratio) redrawn from [37].

V. CONCLUSION

The simple dissolution of surfactant molecules in water makes the micellar media. These media are easy to prepare, but their physico-chemical properties should be known in order to be able to use them correctly in MLC. The two important points to remember are: (i) surfactants adsorb at any interface, and (ii) surfactant associations are dynamic; they form and break more or less rapidly. The first point partly explains the uncommon solute retention and selectivity obtained in MLC due to modifications of the stationary phase by surfactant adsorption. The second point may explain the slow mass transfer often observed in MLC and may be responsible for the reduced chromatographic efficiency.

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3

Historical Development of Micellar Liquid Chromatography

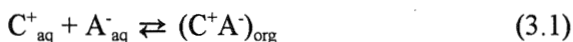
Besides cooking and food processing, surfactant preparation is probably one of the earliest chemical reactions by mankind. The preparation of usable soap was done by heating fresh ashes mixed with animal fat. It may look odd to compare doing laundry with chromatography. However, laundry can be regarded as a separation method: in the process, soaps are used to separate soil and dirt from linen fibers. The history of the development of MLC is not that old. MLC can be considered as the offspring of ion-pair chromatography which appeared in the sixties. MLC itself was introduced as a chromatographic technique using micellar mobile phases in the late seventies by Daniel W. Armstrong [1]. He and others developed and promoted the MLC technique in the eighties. Since it is important to know the basics of ion-pair chromatography to understand the MLC birth and development, the history and principles of ion-pair chromatography are briefly reviewed before the exposition of MLC history.

I. ION PAIR CHROMATOGRAPHY

I.1. Principles

An ion-pair is the association by coulombic forces of a cation and an anion moieties. Besides coulombic forces, the two ions associate because the dielectric constant of the solvent is low and/or because the ions have a lipophilic part. The hydrophobic effect and the electrostatic interaction both contribute to the ion-pair formation. The ion-pairing agent or counter-ion

is most often a strong electrolyte with an alkyl chain of various length. Quaternary ammonium alkyl halides are used as pairing agents for anions. Sodium alkyl sulfates or sulfonates are anionic salts used as pairing agents for cations. The polarity of the ion-pair is much lower than the polarity of each individual ion. Ion-pairing is used to transfer ions from a polar aqueous phase to a nonpolar organic one [2]. In its simplest form, the partition equilibrium can be represented by



where C^+ and A^- are any organic cation and anion, respectively. The ion-pair (C^+A^-) behaves like a polar organic molecule and will therefore preferentially dissolve in a polar organic phase such as an alcohol, a chlorinated solvent or solvent-mixtures such as ether and alcohol or ketone and alkane.

1.2. History and Development of Ion-Pair Chromatography

In 1973, ion-pair formation associated with phase transfer was used by Eksborg and Schill [3] to develop normal-phase ion-pair chromatography. A polar bare silica stationary phase was impregnated with an aqueous ionic solution. The eluent, a mixture of hexane, chloroform and pentanol, could separate various benzoic and salicylic acid derivatives. Karger [4] and Knox [5] used the normal phase mode with impregnated stationary phases to separate catecholamines by ion-pairing.

Soon, Knox thought to use the hydrophobic effect to form ion-pairs in aqueous phases. He developed the first use of reversed-phase ion-pair chromatography in 1976 [6]. He termed it "soap chromatography." He added ionic surfactants to the polar hydro-organic mobile phase. They adsorb on the alkyl-bonded stationary phase. They also associate with the hydrophobic and ionic analytes.

Two mechanisms can explain the analyte retention: (i) A classical ion-exchange mechanism, the ionic solute interacts with the counter-ion covered stationary phase. (ii) An ion-pairing mechanism, the hydrophobic ion-pair partitions with the alkyl-bonded stationary phase. The true

mechanism may depend on the nature of the ions. It is accepted that a mechanism intermediate between ion-exchange and hydrophobic partitioning is likely. The dynamic ion-exchange involves differential adsorption of both the analyte ion and the surfactant counter-ion as described by Melander and Horváth [7].

1.3. *Factors Influencing Retention*

The nature and concentration of the pairing ion and of the organic modifier, the ionic strength, the pH and the character of the stationary phase are the main factors acting on the retention behavior of the solute-ion. Some of these factors are interdependent.

a) Ion-Pair Adsorption on the Stationary Phase

The pairing ion adsorbs on the stationary phase. With hydro-organic mobile phases, Freundlich-type adsorption isotherms were obtained [8]. Figure 3.1 shows the adsorption isotherms of sodium octyl sulfonate, $\text{CH}_3(\text{CH}_2)_7\text{SO}_3^- \text{Na}^+$ (top) and sodium dodecyl sulfonate, $\text{CH}_3(\text{CH}_2)_{11}\text{SO}_3^- \text{Na}^+$ (bottom) on Lichrosorb RP8 sorbent with different acetonitrile (ACN)-water mobile phases. The adsorbed amount of pairing ion increases exponentially with the carbon number in its alkyl-chain. It decreases rapidly with the organic modifier content (Figure 3.2). The ionic solute retention factors are directly proportional to the amount of pairing-ion adsorbed on the stationary phase.

The ion-pair adsorption phenomenon renders the equilibration of the stationary phase critical. Working with nonequilibrated stationary phases will produce nonreproducible results. The time needed to equilibrate a column can be surprisingly long. For example, let's estimate the mobile phase volume needed to equilibrate a 25 cm column containing 2 g of Lichrosorb RP8 ($350 \text{ m}^2/\text{g}$) with a water/ACN 80/20 v/v solution containing 0.005 M sodium dodecylsulfonate. Figure 3.1 (bottom) shows that the ion-pair adsorption amount is $0.8 \mu\text{mol}/\text{m}^2$. In the 25-cm column, the surface area is 700 m^2 , then 560 μmoles of sodium dodecylsulfonate are needed to equilibrate the column. This amount is contained in 112 mL of mobile phase. If the working flow rate is 1 mL/min, the 25-cm column will need at least 112 min or approximately two hours to be correctly equilibrated.

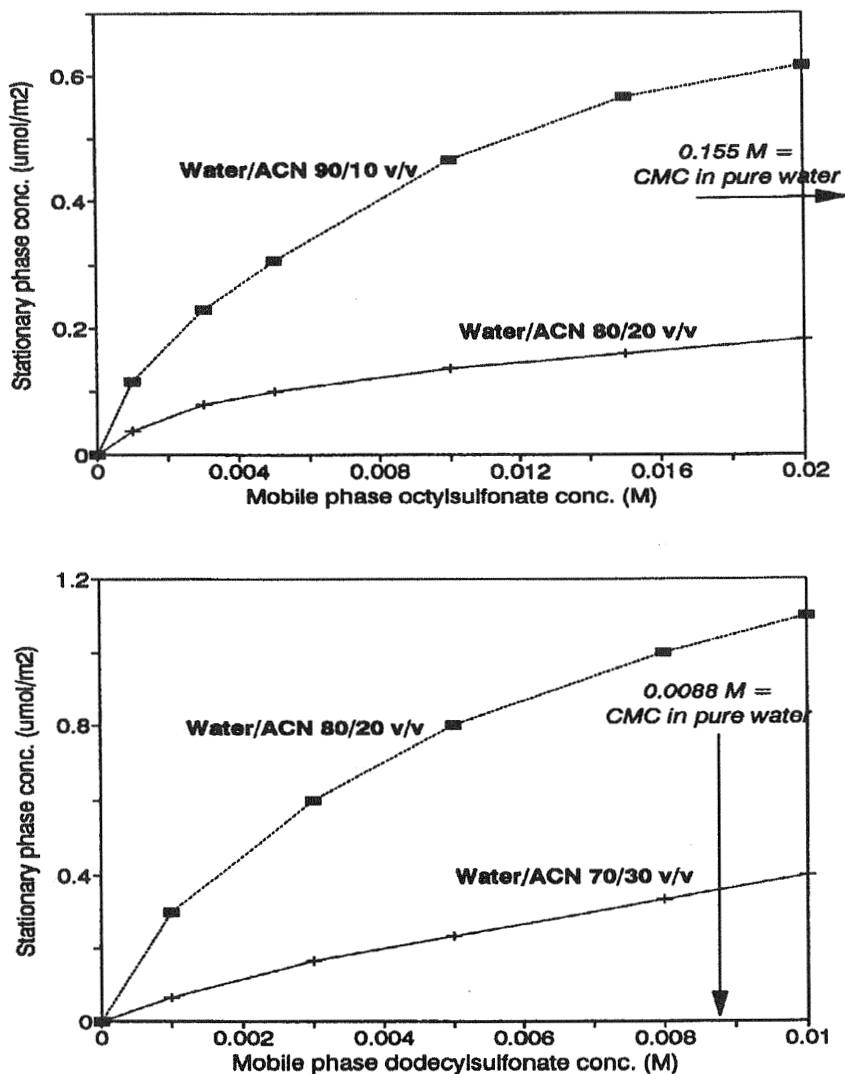


Figure 3.1 Adsorption isotherms on a C8 bonded silica stationary phase. Column 25 cm x 4.6 mm i.d. Lichrosorb RP8, 350 m²/g, 10 μ m particle diameter. Top: sodium octylsulfonate in acetonitrile (ACN)-water mobile phases. Bottom: sodium dodecylsulfonate in ACN-water mobile phases, 20°C, data from Ref. [8].

The ion-pair adsorption on the stationary phase precludes the use of organic modifier gradients that would completely modify the stationary phase adsorbed layer (Figure 3.2).

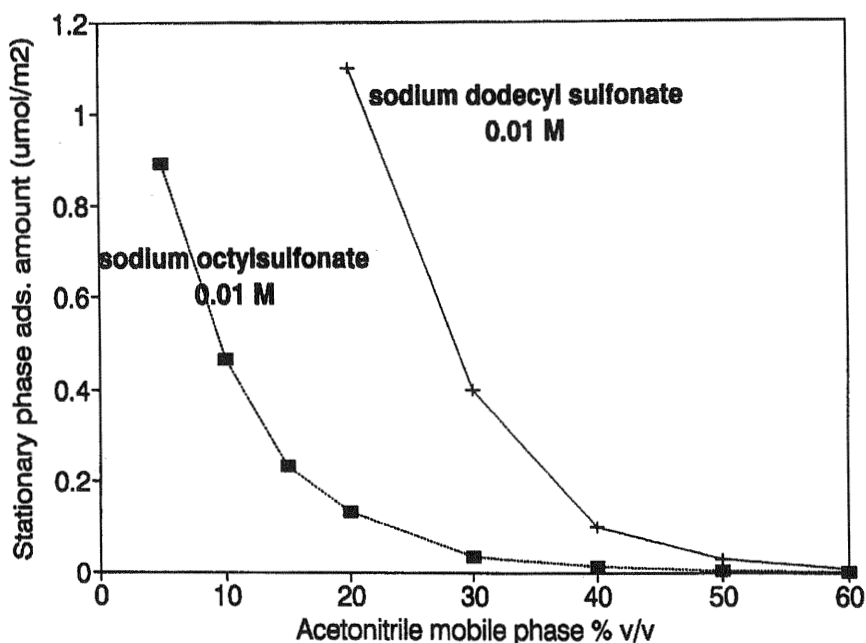


Figure 3.2 Rapid surfactant desorption with the increase of mobile phase organic modifier content, same experimental conditions as Figure 3.1.

b) Solute Structure

In ion-pair chromatography, Tomlinson and Riley [9] have estimated the ion retention factors using functional group values, τ , related to the Hammett constants. This shows that the hydrophobic part of the ionic solutes is responsible for the retention as well as its ionic part. The latter part may be pH and ionic strength dependent.

c) pH Effects

The acidity constant, pK_A , is used to measure the ionization degree of a solute at a given pH value. An acidic solute, HA, is 99% or more in the A^- ionic form for pH values such as

$$pH \geq pK_A + 2 \quad (3.2)$$

Similarly, a basic solute, B, is 99% or more in the protonated ionic form, BH^+ , for the pH values:

$$pH \leq pK_A - 2 \quad (3.3)$$

If both conditions are not fulfilled, the ionizable solute is retained partly in its molecular form and partly through the dynamic ion-exchange process. The retention factor of ionizable compounds shows a sigmoidal dependance on the mobile phase pH. The lower retention of the sigmoid corresponds to the molecular form partitioning. The higher retention values correspond to the dynamic ion-exchange of the ionized form of the solute.

All variations are possible. For example, the retention of the solute 2-chlorobenzoic acid is not sensitive to a pH change on the practical 1-8 range with a quaternary ammonium pairing ion and ACN/water 50/50 v/v mobile phases [9]. Its pK_A value is 2.9 in pure water. The retention factor of the solute 4-aminobenzoic acid plotted versus the mobile phase pH is a sigmoidal curve with a maximum retention variation in the pH zone at approximately pH 4 [9]. The pK_A value of 4-aminobenzoic acid is 4.9 in pure water, but it can be close to 4 in ACN/water 50/50 v/v solutions.

d) Ionic Strength Effects

It is established in ion-pair chromatography that salts always reduce the ion-pair retention factors by shielding ion-ion interactions. The plot of the retention factor, k , of a ionic solute versus the inverse of the mobile phase ionic strength, I^{-1} , is linear with a positive slope [9]. Often, an increase in ionic strength decreases the retention factor and reduces the efficiency of the system. From a practical point of view, the mobile phase salt concentration (ion-pairing agent, solute and buffer) should be maintained below the 0.02 M limit [10].

e) Organic Modifier Nature and Concentration

Methanol, acetonitrile or isopropanol are the organic modifiers commonly used in mixtures with water. Higher organic modifier concentrations produce drastic reduction of ionic solute retention factors. This is due to the increased elution strength of organic rich mobile phase and also to the decrease of the pairing ion adsorption on the stationary phase (Figure 3.2). As illustrated by Figure 3.1, the ion-pair adsorbed amount depends on the nature and concentration of the organic modifier and on the alkyl chain length of the ion-pairing agent itself.

f) Nature of the Ion-Pair Agent

Changing the ion-pair agent is the most readily available way for modifying the ionic solute retention factors. This is achieved by selecting a ion-pair of the same class but with a different alkyl chain length. If the alkyl chain length increases, the ion-pairing agent adsorbed amount increases (Figure 3.1) and the ionic solute retention factors increase. Also the hydrophobic character of the ion-pair increases. Practically, alkyl chain length ranging from 6 methylene units (hexyl) to 16 units (hexadecyl) are available in chemical catalogues.

g) Temperature

An increase in temperature is almost always associated with a decrease of the retention factor [10]. This is again a result of the ion-pairing agent adsorption whose isotherm changes with temperature. This is a problem in the daily use of ion-pairing chromatography. Variations of ambient temperature result in a poor reproducibility of the retention factor values.

Table 3.1 summarizes the parameters acting on the solute retention in ion-pairing chromatography. The big interest of the technique is its ability to analyze and to separate ionic solutes together with molecular solutes using a classical RPLC column and hydro-organic mobile phases with low amounts of the ion-pairing agent. A satisfying long term reproducibility of the results may be difficult to obtain, however.

Table 3.1 Parameters Acting on the Ionic Solute Retention in Ion-pairing Chromatography

Parameter	Effect on retention
<i>Stationary phase</i>	
bonded alkyl moiety	increase
bonded density	increase
<i>Mobile phase</i>	
ion-pairing alkyl chain length	exponential increase
ion-pairing concentration	linear increase
organic modifier concentration	exponential decrease
organic modifier nature	change selectivity
pH	depends on solute pK_A
ionic strength I	decrease with I^{-1}
temperature	decrease

An increase of the listed parameter produces the indicated effect on the ionic solute retention factor.

II. THE BIRTH OF MICELLAR LIQUID CHROMATOGRAPHY

The concentration of surfactant in the solutions used as mobile phases in ion-pair chromatography should not pass the cmc value. It was known that the solute retention was different when micelles were present in the mobile phase [5-10]. Figure 3.3 illustrates the dramatic retention change observed when the surfactant cmc is passed [11]. The retention factor of benzyl trimethylammonium bromide (BTAB), a cationic solute, is plotted versus the amount of SDS adsorbed on the stationary phase (a cyanopropyl bonded phase). The BTAB retention factor is directly proportional to the SDS

adsorbed amount. This typical ion-pairing behavior ends when the SDS concentration reaches the cmc (~ 0.008 M, inset Figure 3.3). The BTAB retention factor drops from ~ 2000 for a SDS ion-pairing concentration just below the cmc to 40 for a SDS concentration of 0.016 M or two cmcs [11]. However, it was not the people working in the ion-pair chromatography field that first developed the use of surfactant solutions above the cmc for chemical separations. Rather, MLC was invented by people studying the use of surfactant solutions as a new and original medium with particular physicochemical properties.

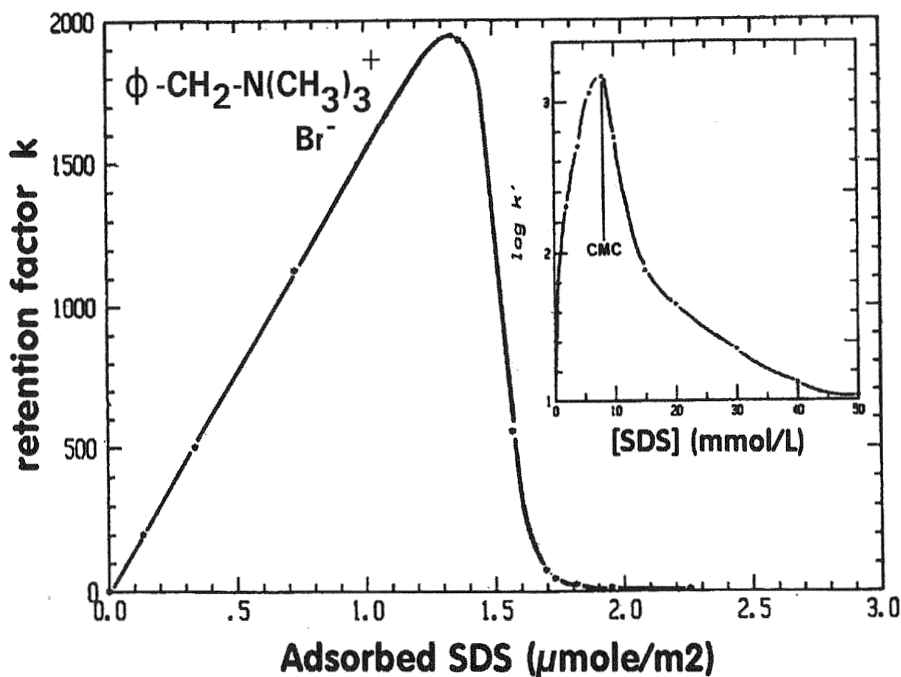


Figure 3.3 Retention factor of the cationic solute benzyltrimethylammonium bromide (BTAB) versus the adsorbed amount of SDS on a CN bonded phase. Column $10\text{ cm} \times 4.6\text{ mm}$ i.d., CPS Hypersil $5\text{ }\mu\text{m}$. Inset shows $\log k$ vs. the SDS concentration in the aqueous mobile phase. Reprinted from Ref. 11 with permission of the American Chemical Society.

II.1. *Gel Permeation Chromatography*

a) A Pioneering Work

In the early sixties Herries and Richards [12] studied the differences in reactivity of protein molecules before and after denaturation. They considered micelles as possible models since surfactants were well-established denaturing agents. Their studies led them to be involved in kinetic studies of reactions where the reactants partition differently between an aqueous and micellar phase. For their calculations they needed the partition coefficient of the various reactant molecules between the aqueous and micellar phase. The gel permeation chromatography (GPC) technique was used for this purpose.

Their idea was: GPC separates the big molecules from the small ones. A micelle is a molecular association big enough to be excluded from the GPC stationary phase gel. The monomer surfactant molecules are small and retained inside the gel pores. The reactant molecules that partition between the micellar and aqueous phase will have a retention volume intermediate between the micelle retention volume (close to the exclusion volume) and the permeation volume. Then, the reactant retention volume can be related to its micellar partition coefficient.

In an article exposing their results [12], they were the first to use the Martin and Synge theoretical derivation to establish the equation relating the solute retention to its micellar partition coefficient. Although their Sephadex G-25 column presented a strong adsorption for some solutes, they were able to obtain straight lines plotting the $V_i/(V_e - V_o)$ parameter versus the SDS surfactant concentration, the subscripts i, e and o refer to the internal (pore) volume of the GPC gel, the solute elution volume and the external (exclusion) volume, respectively. The slope and intercept of the lines allowed them to determine accurately the micellar partition coefficient of the seven compounds studied [12]. They were not interested in the separation ability of the technique and did not go further in this direction.

b) The Deliberate Use of Micellar Phases for Separation

In the late seventies, Daniel Wayne Armstrong was working as a graduate student in the Janos Fendler's group at Texas A&M University (College Station, Texas). His work was related to synthesis and chemical and biochemical reactions in micellar media. To measure the micellar partition coefficient of transfer ribonucleic acids (tRNA), he used the GPC method described by Herries, Bishop and Richards [12]. He got the partition coefficients of the tRNA solutes he was looking for. He readily noticed the separation capability of the method and immediately announced this fact in his very first article on the topic: *The relative low cost, the ease of operation and shortness of time for a complete run merit, we believe, the development of this technique to its full potential* [13].

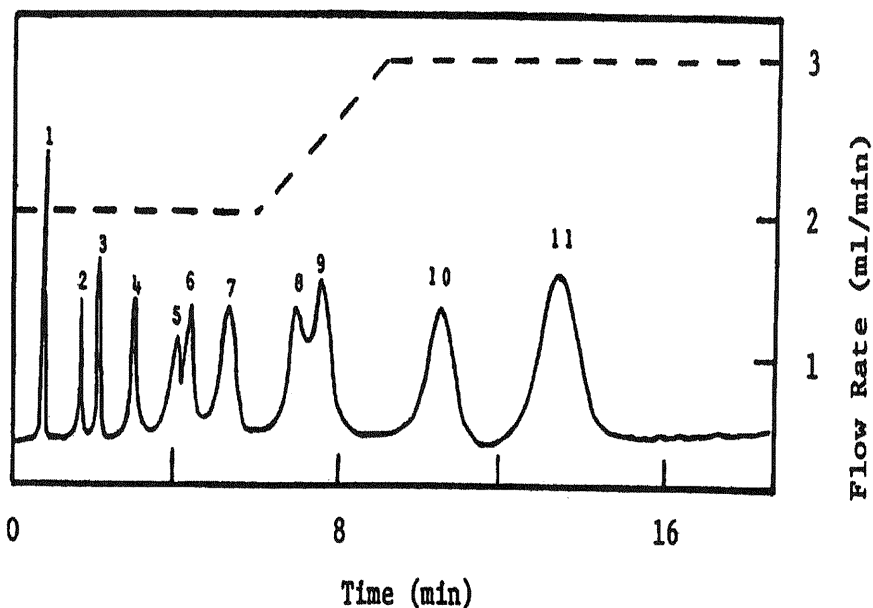


Figure 3.4 The first MLC chromatogram. Mobile phase: 0.2 M SDS, column: 30 cm x 4 mm i.d. Micropack MCH-10, 10 μ m C18 particles, flow rate varying between 2 mL/min and 3 mL/min as indicated by the dashed line. Solutes: a variety of phenols, PAHs and other aromatic compounds. Reprinted from Ref. 16.

II.2. *Thin Layer Chromatography*

After obtaining his Ph.D., Armstrong took an Assistant Professor position at the Bowdoin College (Brunswick, Maine). There, due to the lack of GPC systems, he developed his ideas on the use of micellar phases for chemical separations with another more affordable technique: thin layer chromatography (TLC). He successfully used micellar normal and reversed phases with TLC plates to separate a variety of chemicals [1, 14]. He investigated anionic micellar solutions with SDS, cationic ones with CTAB and also reversed micelles with sodium dioctylsulfosuccinate (Aerosol OT or AOT) reversed micelles in cyclohexane [14]. He already noticed the broadening of the initial spots on the micellar developed plates [1] and thought to use cyclodextrins the same way as micelles [15]. He coined the term: “*Pseudo-phase Liquid Chromatography*” to designate all the chromatographic techniques, GPC, TLC or HPLC, that use a secondary chemical equilibrium, with micelles or cyclodextrins, in the separation process [15].

II.3. *High Performance Liquid Chromatography*

a) Armstrong's Cornerstone Work

In 1980, Armstrong moved to Georgetown University at Washington DC. He started to apply his ideas on the capabilities of micellar phases to HPLC. The first MLC article presented the separation of phenols and PAHs. The first published MLC chromatogram is shown in Figure 3.4 [16]. The efficiency of the o-cresol peak (Peak #7) was only 800 plates for 30 cm ODS column (h.e.t.p = 375 μ m or 38 particle diameters). This low efficiency was probably not pointed out because a flow gradient was used [16].

With Faruk Nome, an ex-member of the Fendler group that was spending some time as a post-doc with Armstrong, they both wrote the cornerstone article establishing the theory of MLC selectivity [17]. They proposed the three-phase model (see Chapter 5) and established it with experiments and a supporting theory based on the following equation:

$$\frac{\phi}{k} = \frac{\bar{v} (P_{WM} - 1)}{P_{WS}} [M] + \frac{1}{P_{WS}} \quad (3.4)$$

where ϕ is the phase ratio, $\phi=V_s/V_o$, the ratio of V_s , the stationary phase volume and V_m , the mobile phase volume inside the column; k is the solute retention factor; $\bar{v}$ is the surfactant molar volume; $[M]$ is the surfactant concentration in the micelle form ($[M]$ = total surfactant concentration minus cmc), and the P_{WM} and P_{WS} constants are the dimensionless solute partition coefficients between the micelles and water (WM) and between the stationary phase and water (WS), respectively. This equation helped to determine the solute micellar partition coefficient and stationary phase affinity [17].

b) The Early Spread of the MLC Technique

Interested by the use of micellar mobile phases, several research teams started to work in the MLC field. Linda Cline-Love, at Seton Hall University (South Orange, NJ) was studying the spectroscopic properties of the micellar media. Armstrong proposed to use these properties to enhance the detection of fluorescent and phosphorescent compounds [18]. Cline-Love further developed such use of the micellar phases [19]. Also, her group verified and confirmed Armstrong's theory [20]. John Dorsey, at University of Florida (Gainesville, FL), was the first to investigate the efficiency problem in MLC [21]. Willie Hinze, another former Fendler student that took an academic position at Wake Forest University (Winston-Salem, NC), showed that nonionic surfactants also obeyed Armstrong's theory (Eq. 3.4) and could be used as well as ionic surfactants [22]. Gordon Kirkbright, at the University of Manchester (England), used MLC to separate salts of dithiocarbamates with cationic surfactants [23].

III. MODERN MICELLAR LIQUID CHROMATOGRAPHY

III.1. 1982-1986, a Growing Interest

a) Worldwide Expansion

After 1982, more and more research groups located worldwide started to investigate the capabilities of MLC. In the US, Armstrong, now a professor at Texas Tech University (Lubbock, TX), was still active in the field [24-25]. Armstrong [26-27] and the other American scientists [28] wrote reviews to increase the diffusion of the technique. Researchers in Italy [29], China [30-31], Spain [32], France [11, 33] or Japan [34-35] increased the knowledge of the foundations of MLC and its application range. The denomination: *Pseudo-phase Liquid Chromatography* was progressively replaced by: *Micellar Liquid Chromatography* [11, 21, 33].

b) The 1986 New York ACS Meeting

Armstrong and Hinze proposed to the American Chemical Society to organize a symposium on the use of *Ordered Media in Chemical Separations* at its 191st Meeting held in New York in April 1986. A high percentage of the people working in the MLC field attended or were invited. With the texts of the conferences, the two symposium organizers edited a book containing a great part of the MLC knowledge at this time [36]. It is important to point out that Armstrong himself gave a talk on cyclodextrin (CD) complex formation. He was happy that people used and developed further the MLC technique he invented, but he thought that the technique had limited applications. The use of CDs for chemical separations was already his main research interest. He was so successful applying the CD chemistry to the difficult task of chiral separations that so far, he never went back to work in the MLC field.

The MLC limitations were clearly seen during this symposium when the capabilities of the micellar phases associated with the then new capillary electrophoresis technique were exposed. Electropherograms showing efficiencies in the 100,000 plates/m range were displayed [37].

III.2. 1987-Present, Mature MLC

After the 1986 symposium, it became clear that MLC would not supplant any separation methods. However, the interest of the scientific community was still significant as shown by the number of MLC publications appearing per year (Figure 3.5). MLC was seen as an interesting technique that deserved studies in two main directions:

- (i) the fundamental way: eluent strength, selectivity and efficiency and
- (ii) the applied way: hydrophobicity studies and original separations.

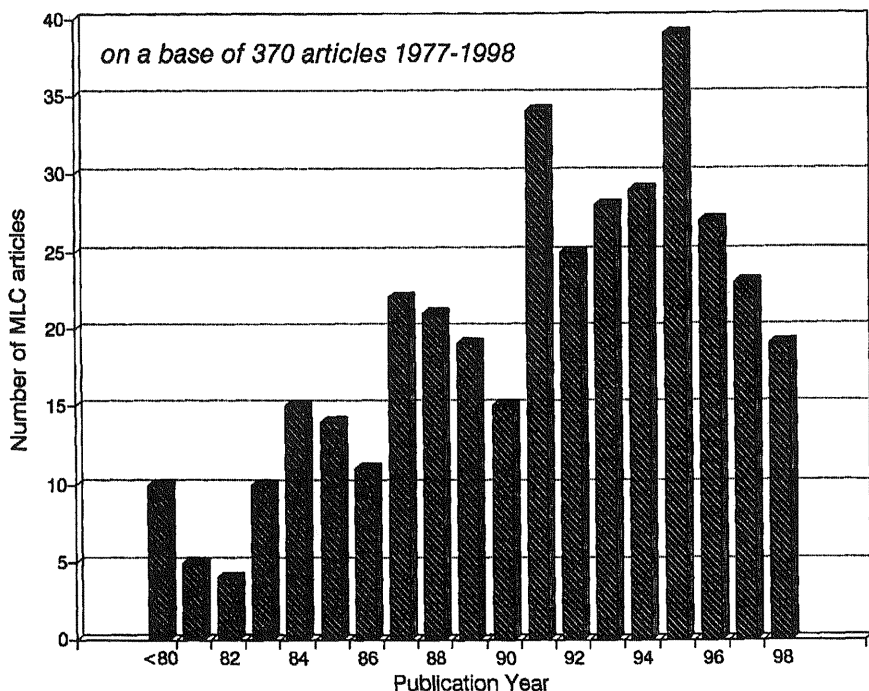


Figure 3.5 Number of articles entirely dedicated to MLC sorted by publication year from 1977 to 1998.

a) Fundamental Studies

Eluent Strength, Selectivity and Efficiency. The very low efficiency obtained with purely aqueous micellar phase was greatly improved by the 3% v/v propanol addition recommended by Dorsey [21]. The relatively weak eluent strength of the aqueous micellar phases was increased also by the addition of alcohols. These studies were initiated by Morteza Khaledi, Dorsey's student [38-39]. Hinze showed that the retention of homologous compounds did not obey the established RPLC models (log k proportional to homologue number) [40]. He proposed a direct transfer mechanism between the micelles and the stationary phase [41]. The original selectivity obtained in MLC were thoroughly investigated by Khaledi [42-43], Cline-Love [44] and others [45]. The reasons for the low efficiency observed in MLC were investigated by Berthod and Hinze [46-47].

Modeling and Optimization. Fundamental studies increased the knowledge of the micellar retention mechanisms. It was then possible to propose theoretical models that allowed chromatographers to predict and optimize their MLC separations [48-50].

b) MLC Applications

Biological Separations. As early as 1985, Cline-Love showed that the surfactant molecules contained in the micellar mobile phase were able to bind to proteins and to reduce greatly their adsorption on the silica based stationary phases. The direct injection of biological samples such as serum or urine samples can be done in MLC without protein precipitation inside the column [51]. This property initiated a great interest for MLC and a high number of works in this area [32, 52-55].

Hydrophobicity and Quantitative Structure Retention Relationship. The micellar partition coefficient of a solute can be related to its hydrophobicity. These partition coefficients are very useful data for the Quantitative Structure Retention Relationship (QSRR) studies. This research field was investigated by numerous groups [56-59].

Separations of Ions and Inorganic Species. The aqueous phase of the micellar mobile phase can dissolve polar solutes and ions. By ion-exchange

or hydrophobic mechanisms, the micellar phase may induce ion separation. Anions [60] as well as cations [61-62] were successfully separated by MLC.

To conclude this chapter, it is important to note that liquid chromatography is certainly not the only technique that can use micelles for separation purposes. Only 5 chapters of the 16 available in the Hinze and Armstrong book [36] dealt strictly with MLC (less than one-third of the book). From the 11 remaining chapters, 6 included cyclodextrin based separations; one dealt with the use of micellar phases in capillary electrophoresis [37] and the last 4 chapters included different nonchromatographic ways to use micelles in separation. For comparison, in 1997, *Journal of Chromatography* published a special volume on the use of micelles for chemical separations [63]. From the 21 contributed articles of this special issue, 5 dealt with MLC, 4 partially discussed MLC results and the remaining 12 were on capillary electrophoresis or micellar electrokinetic chromatography. Again one-third of the special issue only was devoted to MLC. If it is clear that micellar phases are most appropriate for separations with the capillary electrophoresis technique, it shows that the MLC technique is still useful in numerous occasions.

It is the purpose of this book to review as deeply as possible the subject of micellar liquid chromatography. All the studies presented in this historical chapter are developed in dedicated chapters in the book. Most of the recent work and applications developed in the nineties were not cited in this chapter; they are discussed in detail in their respective chapter.

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The Role of the Stationary Phase

I. INTRODUCTION

The two main properties of surfactant molecules are micelle formation and adsorption at interfaces. In Micellar Liquid Chromatography (MLC), the micelle formation property is linked to the mobile phase. Micelles play the role of the organic modifier in RPLC. Nonpolar solutes partition themselves between the micelle apolar core and the apolar bonded stationary phase. This partitioning will be the subject of Chapter 5. The surfactant adsorption property is linked to the stationary phase. A significant number of surfactant molecules may adsorb on the stationary phase surface changing its properties. The study of such adsorption and its associated problems is the main subject of this chapter.

II. STATIONARY PHASE CHARACTERIZATION

Although new materials are rapidly emerging for chromatographic stationary phases, silica is still the principal medium used as the material for liquid chromatography (LC) packing [1-3].

II.1. Physicochemical Properties of Silica

The three physicochemical parameters for silica relevant to chromatography are the particle size, the surface area and the porosity. The other important parameters are the particle shape, the carbon percentage, the ligand density and bonding structure and the surface silanol concentration [1].

Table 4.1 Physicochemical Properties of Selected Silica-Based HPLC Packings

Trade name and manufacturer ^a		Particle		Surface area m ² /g	Pore diam. nm	Pore volume cm ³ /g	Bonding nature ^c	C load %C	Bonding density μmol/m ²
		size μm	shape ^b						
Accusphere	J&W	5, 10	O	210	12	0.6	C8	7	3.1
Adsorbosphere	Alt	5	O	200	8	0.4	C18 m ec	12	3
Alphabond	Alt	5	O	300	12.5	0.9	C18 m	10	1.7
Apex ODS-2	Jon	5, 10	O	170	10	0.8	C18 m ec	11	3
Bakerbond	JTB	5	K	200	15	0.75	C18 m ec	15	3.9
Bio-Sil C18	Brd	3, 5	K	300	10	0.75	C18 m ec	19	3.5
Cosmosil C8	Phe	5, 10	O	330	11	0.9	C8 ec	12.5	3.9
Cosmosil C18	Phe	5, 10	O	330	11	0.9	C18 m ec	20	3.5
Cosmosil CN	Phe	5, 10	O	330	11	0.9	C3CN ec	8	4
Hypersil	Lif	3, 5	O	150	12	0.5	unbounded	0	0
Hypersil SAS	Lif	3, 5	O	104	12	0.4	C1 m ec	2.6	4.5
Hypersil MOS	Lif	3, 5	O	129	12	0.4	C8 m ec	7	4.1
Hypersil ODS	Lif	3, 5	O	105	12	0.4	C18 m ec	9.5	2.9
Hypersil CPS	Lif	3, 5	O	115	12	0.4	C3CN m	4.2	3.6
Kromasil C18	EkN	5-15	O	340	10	0.9	C18 ec	19	3.1
LiChrosorb RP	EMS	5, 10	K	550	6	0.8	C18 ec	12	2.5

Table 4.1 Continued

LiChrospher Diol	EMS	5, 10	O	350	10	1.1	OH m	8.3	4
LiChrospher RP8	EMS	5, 10	O	350	10	1.1	C8 m	12.5	4.1
LiChrospher RP18	EMS	5, 10	O	350	10	1.1	C18 m	21.5	3.9
LiChrospher NH ₂	EMS	5, 10	O	350	10	1.1	NH ₂ m	4.5	3.8
Nucleosil 100	Mng	5, 10	O	350	10	1.0	unbounded	0	0
Nucleosil C8	Mng	5, 10	O	350	10	1.0	C8 m	8	2.1
Nucleosil C18	Mng	5, 10	O	350	10	1.0	C18 m ec	14	2
Partisil C8	Wht	5, 10	K	350	8.5	0.7	C8 m ec	9	2.5
Partisil ODS	Wht	5, 10	K	350	8.5	0.7	C18 polymeric	10.5	-
Resolve C8	Wat	5	O	180	9	0.5	C8 m	6	2.6
Resolve C18	Wat	5	O	180	9	0.5	C18 m	10	2.8
Spherex C1	Phe	5, 10	O	180	10	0.8	C1 m	3	4.5
Spherex C18	Phe	5, 10	O	180	10	0.8	C18 m ec	11	3
Spherex phenyl	Phe	5, 10	O	180	10	0.8	phenyl	3	1.3
Spherisorb ODS	PhS	3, 10	O	220	8	0.5	C18 m ec	12	2.7
Spherisorb phenyl	PhS	3, 10	O	220	8	0.5	phenyl m	3	1
Symmetry C18	Wat	5	O	330	12.5	1.0	C18 ec	18	3.3
Ultracarb ODS	Phe	5, 10	O	550	6	0.8	C18 m ec	31	4.1
Ultremex C18	Phe	5, 10	O	200	8	0.8	C18 m ec	13	3.3

Table 4.1 Continued

Zorbax C8	DuN	5, 7	O	330	10	0.8	C8 m ec	10	3
Zorbax ODS	DuN	5, 7	O	330	7	0.6	C18 m ec	20	3.5
Zorbax phenyl	DuN	5, 7	O	200	8	0.4	phenyl m	4	1.6

a)- Manufacturers are: Alt=Alltech Associates, Deerfield, IL; Brd=BioRad Lab., Bruxelles, Belgium; DuN=Dupont de Nemours, Wilmington, DE; EkN=Eka Nobel, Surte, Sweden; EMS=E. Merck Sciences, Gibbstown, NJ; J&W=J&W Scientific, Folsom, CA; Jon=Jones Chromatogr., Hengoeg, Mid Glamorgan, UK; JTB=J.T. Baker, Phillipsburg, NJ; Lif=LIFE Science Int., Runcorn, Cheshire, UK; Mng=Macherey Nagel, Dueren, Germany; Phe=Phenomenex, Palos Verdes, CA; PhS=Phase Separations, Queensferry, Clwyd, UK; Wat=Waters Corp., Milford, MA; Wht=Whatman, Maidstone, Kent, UK.

b)- Particle shape: O=spherical; K=irregular.

c)- C1=trimethylsilane; C8=dimethyl octylsilane; C18= dimethyl octadecylsilane, C3CN=dimethyl cyanopropylsilane, phenyl=dimethyl phenyl silane; m=monomeric, ec=end-capped

Data compiled from Refs. 1, 3 and 6.

a) Particle Size

Silica packings are always obtained with a particle distribution, *i.e.*, a stated 5- μm particle diameter is an average diameter. The polydispersity of a silica powder is related to the width of the particle size distribution. It can be given by the ratio d_{90}/d_{10} , particle diameters of which 90% and 10% of the particle are smaller than or equal to, respectively. The ratio d_{90}/d_{10} should be as close to unity as possible (monodisperse silica). Particle size is always associated to particle shape which can be spherical or irregular. Table 4-1 lists the particle size and shape of some commonly used commercial silica-based LC packings.

b) Surface Area

The amorphous silica used for LC packings is highly porous. The specific surface area of a silica sample is equal to the sum of its internal (pores) and external areas [4]. The specific surface area, $\bar{A}$, of a porous silica sample is expressed in m^2/g . $\bar{A}$ is due to internal area at more than 99% [1]. It is easily measured by the BET method that uses gas adsorption isotherms. Surface area and porosity are linked.

c) Pore Size and Pore Volume

Pores are holes, cavities and/or channels communicating with the silica surface. Pores can be regarded as cylinders with a diameter, p_d , usually measured in nm, sometimes in $\text{\AA}$ ($=0.1 \text{ nm}$). Their total volume is the pore volume, V_p , measured in cm^3/g or mL/g of material. The pore size distribution is measured by the mercury intrusion method [1, 4]. A good chromatographic silica packing should have a narrow pore size distribution. In such case, pore volume, pore diameter and specific surface area can be linked by the approximative relation:

$$\bar{A} = 4000V_p/p_d \quad (4.1)$$

with the units m^2/g , cm^3/g and nm for $\bar{A}$, V_p and p_d , respectively. Eq. 4.1 can be used to estimate the pore volume of a given silica packing when the surface area and pore diameter are given. The typical pore size of classical LC packing ranges between 8 and 20 nm (80-200 $\text{\AA}$) (Table 4.1). Special wide pore packings with 30 to 100 nm (300-1000 $\text{\AA}$) pore size are marketed

and could be useful in MLC to avoid any micelle size exclusion. The micelle diameter is in the 2 to 5 nm range (20-50 Å) (Chapter 2).

II.2. Porous Silica for LC Column Packings

a) Efficiency, Pressure and Retention Factors

Particle size is one of the most important parameters acting on efficiency. The smaller the LC packing particle size, the higher is the efficiency [5]. The plate height, H , of a well-packed column can be as low as twice the particle diameter. A 10 μm plate height (100,000 plate/m) should be obtained when 5 μm particles are used to pack a column.

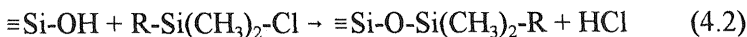
Unfortunately the particle size affects quadratically the column permeability. When the packing particle size is divided by two, the column driving pressure must be four times higher in order to obtain the same flow-rate. Often, very small particle size is associated with short columns in order to reduce the working pressure.

The packing surface area affects the solute retention factors. A high surface area produces a packing which can interact strongly with the injected solutes. The retention factors will be higher than those obtained with another packing with a low surface area of similar nature and polarity.

b) Surface Silanols, Bonding and Polarity

The surface of porous silica is covered by hydroxyl groups called surface silanols (Si-OH) [1, 4]. Silanol groups are responsible for the polarity of the silica surface. They can ionize, the silanol pK_A being 9.8. They are responsible for the silica dissolution in basic ($\text{pH} > 8$) solutions. Naked silica packings are too polar to be used in RPLC. They are used in normal phase LC with apolar mobile phases. To obtain less polar packings for RPLC, it is necessary to derivatize the polar silanol groups.

The silanol groups can react with a number of chemicals. For example, chloro-silanes can be used to bond an R group on the silica surface according to:



Apolar surfaces are obtained with alkyl R groups. A variety of R-groups can be bonded on silica surfaces producing chromatographic packings with a wide range of polarity and properties.

A bonded silica packing is characterized by its carbon load, %C, its surface coverage in $\mu\text{mol}/\text{m}^2$, and the bonding type, monomeric or polymeric, end-capped or not [1, 3, 4]. It was shown that the maximum bonding coverage density was $4.5 \mu\text{mol}/\text{m}^2$ for monomeric dimethyl-alkyl-silyl groups. A lower coverage density means that some silanol groups remain attainable to the solutes. They are called "residual silanols." They are responsible for the tailing observed on the peaks of basic compounds and for the pH sensitivity of the packing. Table 4.1 lists the physicochemical properties of a selection of commercially available HPLC packings.

III. SURFACTANT ADSORPTION

A classical 4.6 mm i.d. LC column contains about 1 g of packing material per 10 cm of column length. The packing surface area is much higher, in the hundredth of m^2 range, than the tubing surface area (a few cm^2). Surfactant adsorption on the porous packing should be considered since it may change the stationary phase surface properties.

III.1. Measurement of Surfactant Adsorption Isotherms

Several methods can be used to estimate the amount of surfactant molecules adsorbed on the stationary phase surface in a LC column. The titration, the breakthrough and the stripping methods will be described because they can produce reliable results with common chromatographic equipment. The results obtained with a given method can be used to check and to corroborate the results of another method. Accuracy of the method depends on the exact knowledge of the column dead volume which should be the first parameter to establish.

a) Dead Volume Measurements

The determination of the void or dead volume of a column is too often neglected by chromatographers. The dead volume, V_0 , is the mobile phase volume inside the column. It corresponds to the inter-particle volume, V_i ,

plus the porous or intra particle volume, V_p . It is also the mobile phase volume needed to elute a nonretained solute.

$$V_O = t_O F \quad (4.3)$$

where t_O is the retention time of a nonretained solute and F is the flow rate. A nonretained solute in RPLC with polar mobile phases can be pure acetone or methanol or a UV absorbing ionic compound such as sodium nitrate or uracil [7]. The dead volume obtained should be compared to the column internal volume, V_C :

$$V_C = \pi/4 d_c^2 L \quad (4.4)$$

where d_c is the column internal diameter and L , the column length. For the popular 4.6 mm i.d. tubing, the column internal volume is 0.166 mL per column cm (~ 1.7 mL for 10 cm).

The ratio V_O/V_C is the total porosity of the column. It is a dimensionless number that should be somewhere between 0.7 and 1. If the V_O/V_C ratio is found to be higher than unity, it means the dead volume is overestimated: the solute used was retained. If the V_O/V_C ratio is found to be lower than 0.7, the dead volume is too small. The test solute was probably excluded from the internal pore volume of the stationary phase. A practical rule of thumb for the 4.6 mm i.d. column only is [7]:

$$V_O \text{ (mL)} \approx 0.1 L \text{ (cm)} \quad (4.5)$$

A 10 cm 4.6 mm i.d. column should have a dead volume close to 1 mL.

For adsorption isotherm determination, the accurate knowledge of the dead volume is required. In most cases, the dead volume tracer method produces appropriate results. When a cross-check of the dead volume value is necessary, the *density method* can be used. First, the column is carefully flushed and filled with methanol which has a density of 0.7914 g/cm^3 at 20°C . It is disconnected from the pump and its two sides are closed to avoid evaporation. The column is weighted. Its mass is W_{MeOH} . Next, the column is similarly flushed and filled with chloroform, $d=1.483 \text{ g/cm}^3$ at 20°C , and weighted. The second mass, W_{CHCl_3} , higher than the first one, allows to obtain the dead volume with a high accuracy using:

$$V_O = 1.446 (W_{\text{CHCl}_3} - W_{\text{MeOH}}) \quad (4.6)$$

The 1.446 factor is only valid for methanol and chloroform at 20°C. Any pair of solvents with a significant density difference and compatibility with the stationary phase can be used instead of methanol and chloroform. The 1.446 factor is trivially the reverse of the solvent density difference.

b) Surfactant Titration

It is often necessary to determine the surfactant concentration in a given solution. Ionic surfactants can be determined by the two-phase liquid method. Ionic dyes are soluble in aqueous phases. They form ion-pairs with surfactant molecules bearing an opposite charge. The ion-pairs are apolar and can be extracted into an apolar organic phase. Chloroform is commonly used.

The typical protocol for titration of a cationic surfactant, *e.g.*, cetyltrimethylammonium bromide (CTAB), is as follows. A known volume of the cationic surfactant solution to be titrated is introduced in a test-tube. It is diluted with water. A 10 mL volume of chloroform is added along with few drops of a cationic indicator dye and a few drops of an anionic dye. Sunset yellow, mordant red 72, disulfine blue VN 150 or alizarins are examples of anionic dyes. Cationic dyes can be dimidium bromide, methyl red, neutral red or chromoxan green. The color of many indicators is pH dependent, and it may be necessary to add a buffer (or an acid) in the aqueous phase. Few cationic surfactant molecules and the anionic dye associate forming ion-pairs that are extracted into the chloroform phase coloring it. A known SDS solution, say, exactly 0.05 M, is added drop by drop. The tube is shaken. An emulsion forms that needs a minute to separate because it is stabilized by the cationic surfactant. SDS molecules and the cationic surfactant form ion-pairs that are extracted into the chloroform phase. As the titration progresses, the emulsion separates faster and faster because there is less and less cationic surfactant to stabilize it. When the endpoint is reached, the SDS molecules in excess displace the anionic dye-cationic surfactant ion-pair and form an SDS-cationic dye ion-pair extracted by the organic phase. This produces a sharp color change of the phases. The equivalent volume of the SDS solution allows to calculate the cationic surfactant concentration in the titrated volume. The protocol for

an anionic surfactant is similar. A CTAB standard solution is used for the titrating agent.

Nonionic surfactant cannot be titrated by this method. They are often compounds with very low volatility. Their concentration can be determined by weighting the remaining solid matter after complete solvent evaporation.

c) The Breakthrough Method

The accuracy of the breakthrough method depends on the ability to detect the surfactant in the mobile phase. A refractive index (RI) detector is most often required since MLC usable surfactants should not absorb UV light. An evaporative light scattering (ELS) detector can also be used successfully since most surfactants are solids at room temperature.

A thermostated chromatographic system should be used. The column, packed with the studied stationary phase, is first equilibrated by passing the aqueous phase, pure water or buffer or water and organic modifier, without any surfactant molecule. The eluent is then switched to a mixture made of the same mobile phase containing a known concentration, C , of surfactant. A surfactant concentration front thus moves down the column. If the surfactant is adsorbed by the packing material, the breakthrough volume of the front, V_B , will exceed the dead or void volume, V_O . The amount of surfactant adsorbed will then be $(V_B - V_O)C$ [8]. This amount should be expressed preferably in $\mu\text{mol}/\text{m}^2$ for convenient comparison between phases.

It should be pointed out that the breakthrough volume may not be easy to determine. Figure 4.1 shows the ideal breakthrough shape and some curves that may be obtained depending on the column efficiency. A progressive surfactant adsorption may be due to pore size polydispersity of the stationary phase: the larger pores are filled before the smaller pores. Also, successive surfactant layers may form. These circumstances may produce slow baseline changes or steps in the detector response. In case of steps, the V_B volume should be corrected. The adsorbed amount of surfactant corresponds to the area shown in Figure 4.1. With an RI detector, the signal is proportional to the surfactant concentration. The recorder trace can be used to correct the V_B volume. The response of most ELS detectors

is *not* proportional to the surfactant concentration. A calibration must be performed for each case or the titration control should be use.

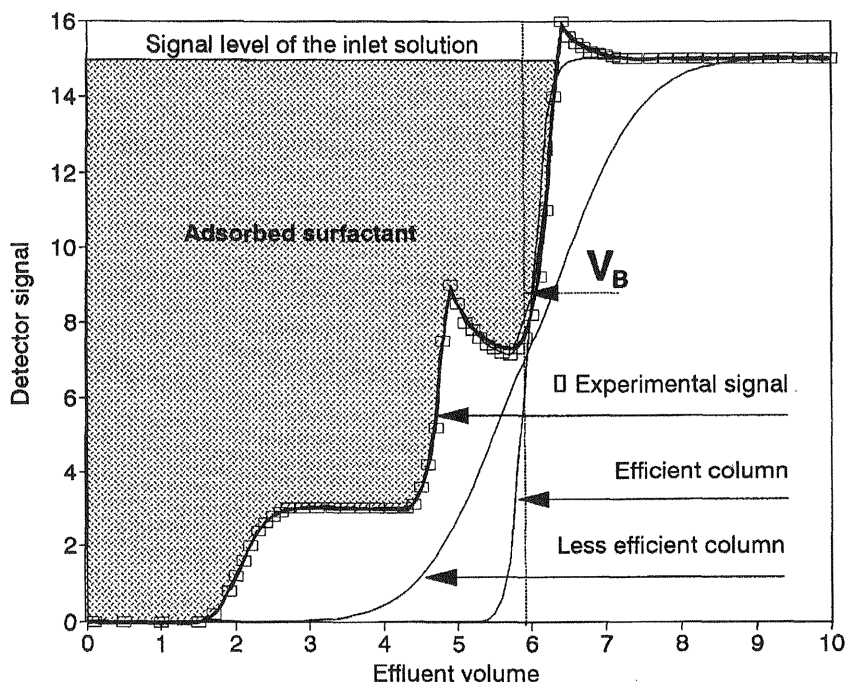


Figure 4.1 Possible detector signal obtained during breakthrough experiments. An efficient column gives a sharp rise of the detector signal. A less efficient column gives a smooth detector rise. The shaded area shows the detector changes obtained in a real study [10] and corresponds to the adsorbed surfactant amount.

For accurate results, it is advisable to control the surfactant adsorption performing a titration. The effluent leaving the column is collected until the detector response reaches a plateau corresponding to the absorbance value of the initial surfactant solution. The surfactant concentration in the collected effluent is determined by titration. The missing surfactant mass is adsorbed on the stationary phase in the column. The mass obtained by titration should corroborate the one obtained using the breakthrough volume.

d) The Stripping Method

Another way to determine the amount of surfactant adsorbed on the stationary phase of a column is to strip it off with a strong eluent and to titrate the collected effluent. This method assumes that there is no irreversible surfactant adsorption. This is not always the case. Irreversible adsorptions were mentioned by Knox for anionic surfactants [9] and by Hinze [10] for a nonionic surfactant. However, in most cases, adsorbed surfactant can be fully stripped off the stationary phase by pure methanol, a mixture of methanol and propanol or pure propanol.

The protocol is straightforward. After complete column equilibration with the surfactant solution at concentration C , the column is flushed by two or three void volumes of aqueous phase without surfactant. This effluent is not collected. This step washes out the water soluble surfactant contained in the void volume without desorbing the surfactant of the stationary phase. Next, the column is rinsed with at least 10 column void volumes of pure methanol (or propanol). The effluent, containing the desorbed surfactant, is collected. Methanol is evaporated and the dry surfactant is re-dissolved in a measured volume of water. The amount of surfactant is determined by titration.

III.2. Adsorption Isotherms with Aqueous Phases

a) Anionic Surfactant

Berthod studied the adsorption of SDS on five different Hypersil® stationary phases [11]. Five 10 cm x 4.6 mm i.d. LC columns were specially selected for the study. All experiments were done in a thermostatic bath at 30°C. The amount of SDS adsorbed on the phases was determined by cross-checking the results obtained with (i) the breakthrough method, (ii) controlling the concentration of the effluent solution, and (iii) by the stripping method. The 3 results were in agreement within an 8% experimental error. Figure 4.2 presents the SDS adsorption on bare Hypersil®, CPS Hypersil®, a cyanopropyl bonded phase, SAS Hypersil®, a methyl bonded phase, MOS Hypersil®, an octyl bonded phase and ODS Hypersil®, an octadecyl bonded phase. The physicochemical properties of these phases are listed in Table 4.1. A very differing behavior was obtained

with nonmicellar mobile phases and micellar mobile phases. Figure 4.2 can be compared with the adsorption isotherms obtained in ion-pair chromatography (Figure 3.1 and Chapter 3) [9].

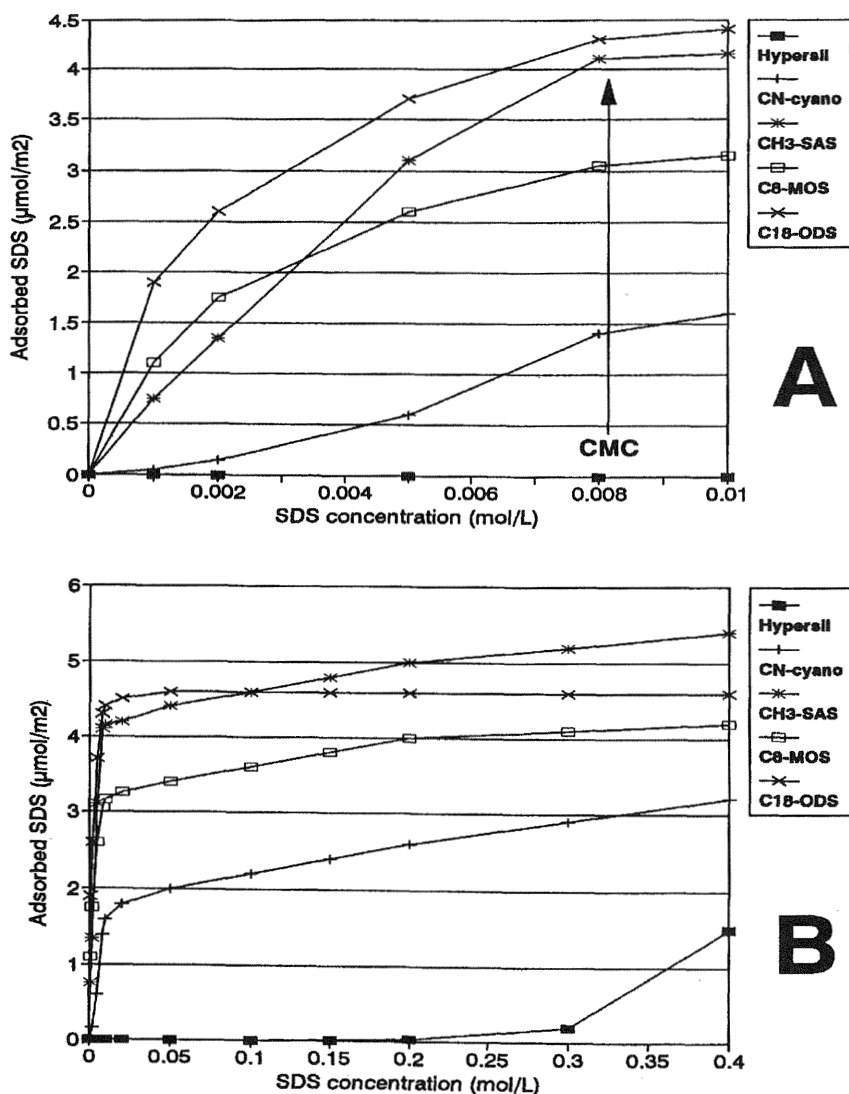


Figure 4.2 SDS adsorption isotherms at 30°C on five different Hypersil® phases. A) non micellar mobile phases; B) micellar mobile phases, data from [11].

Non-Micellar Mobile Phases. Figure 4.2A shows the low concentration part of the adsorption isotherm. From an experimental point of view, the breakthrough volume of mobile phase can be very large. The $1.9 \mu\text{mol}/\text{m}^2$ SDS amount adsorbed on the ODS phase with a 0.001 M SDS mobile phase corresponds to 0.0002 moles of SDS because the ODS surface area is $105 \text{ m}^2/\text{g}$ (Table 4.1). This amount of SDS is contained in 200 mL of mobile phase (=breakthrough volume). It means the duration of the experiment at this point is at least 4 hours at $1 \text{ mL}/\text{min}$.

The cmc is indicated by an arrow. The three nonpolar phases, SAS, MOS and ODS Hypersil®, show a Langmuir-like adsorption isotherm with a concave shape or L type in the Giles' classification [12]. The polar CPS phase shows a convex shape or S type cooperative adsorption. The amount of adsorbed surfactant increases in the order: bare silica < CPS < SAS < MOS < ODS. This is exactly the decreasing order of the stationary phase polarity. No adsorption of SDS on the bare silica was observed in this low concentration range [11]. Above 0.004 M , the SDS adsorption on SAS Hypersil® passes the one on MOS Hypersil®. Hydrophobic interactions are mainly responsible for SDS adsorption in sub micellar solutions.

Micellar Mobile Phases. Figure 4.2B shows the adsorption isotherm on the $0\text{--}0.4 \text{ M}$ SDS concentration range. The four bonded phases have adsorption isotherms of the H-type [12]: the amount of adsorbed surfactant increases rapidly and reaches a plateau for surfactant concentration higher than the cmc. The adsorption plateaus for the alkyl-bonded phases (SAS, MOS and ODS) are very close to each other in the $4\text{--}5 \mu\text{mol}/\text{m}^2$ range. They can be compared to the bonding density of the phases (4.5 , 4.1 and $2.9 \mu\text{mol}/\text{m}^2$, respectively, Table 4.1). Only the ODS plateau seems horizontal. For the other phases, the adsorbed amounts of SDS keep increasing slightly above the cmc. The adsorbed amount of SDS on the CPS phase increases by 70% between 2 cmc (0.016 M) and 50 cmc (0.4 M). The corresponding increases for SAS and MOS phases are only 29% and 31%, respectively.

According to Freundlich, the $\log C_s$ versus $\log C_m$ plot, with C referring to the surfactant concentration and the subscripts s and m referring to the stationary and mobile phase, respectively, should be linear for a Langmuir isotherm. Figure 4.3 shows the Freundlich plots of the five adsorption isotherms. For the 4 bonded phases, there are clearly two

different slopes separated by the micellization area ($\log \text{cmc} = -2.1$). The increase of adsorbed SDS is much lower with micellar solutions than with pre-micellar solution. There is still a small adsorption increase. It is possible to speak of saturation only with the ODS phase. These results were coherent with Dorsey's work on an Altex ODS phase [13]. A different result was observed by Wirth who noted a sharp adsorption maximum for an SDS concentration of 0.007 M, just before the micellization concentration [14-15]. The substrate was a mono-crystalline silicon wafer that was attacked by nitric acid to produce an optically flat silica plate that was derivatized by chlorodimethyloctadecylsilane and end-capped. The bonding density was $2.8 \mu\text{mol}/\text{m}^2$, similar to the ODS Hypersil® ($2.9 \mu\text{mol}/\text{m}^2$). This flat substrate is difficult to compare with porous silica used for LC packings.

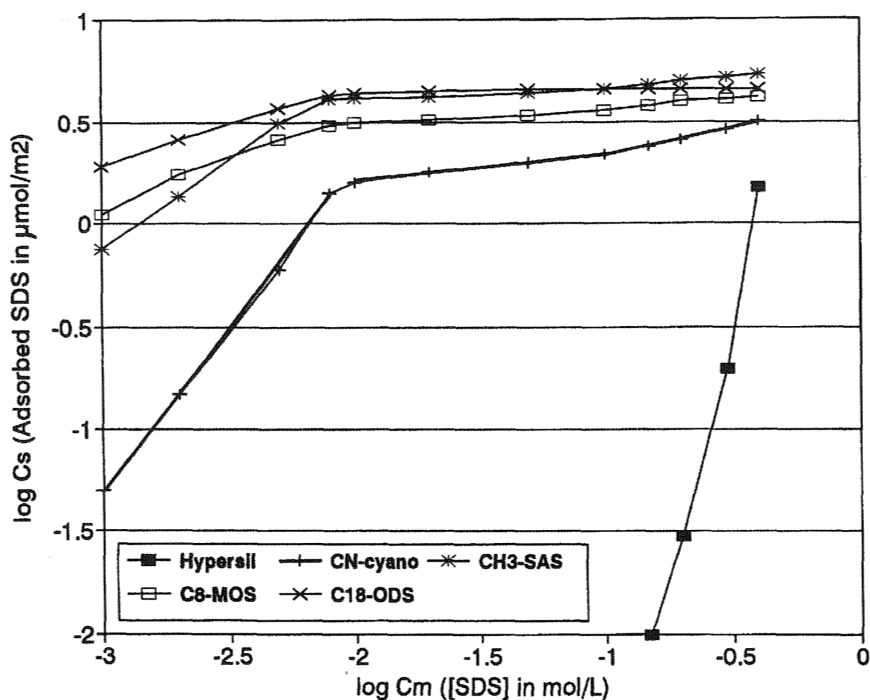


Figure 4.3 Stationary phase versus mobile phase SDS concentrations in log-log coordinate showing a clear break for the cmc value ($\text{cmc} = 0.0082 \text{ M}$, $\log \text{cmc} = -2.1$).

b) Cationic Surfactant

The adsorption of CTAB was similarly studied on the five Hypersil® stationary phases listed in Table 4.1 [11]. The results are presented in Figures 4.4 and 4.5.

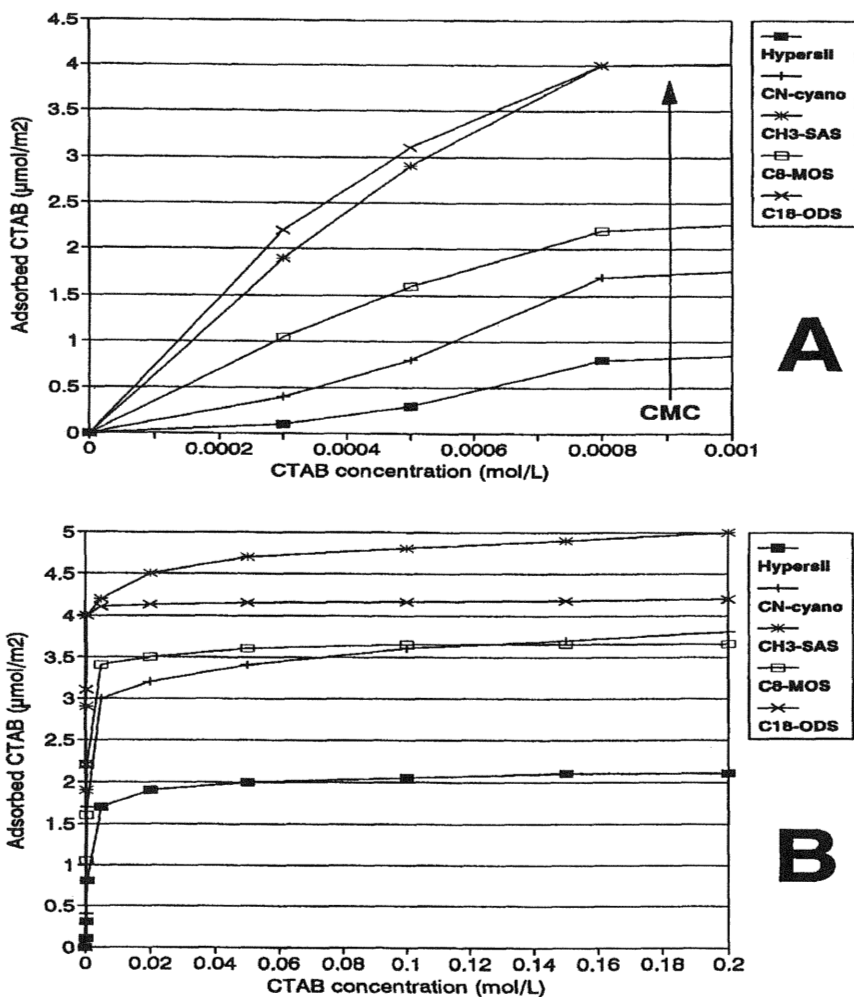


Figure 4.4 CTAB adsorption isotherms at 30°C on five different Hypersil® phases. A) nonmicellar mobile phases; B) micellar mobile phases.

Non-Micellar Mobile Phases. Figure 4.4A shows the CTAB adsorption for sub-micellar solutions. The conclusions are exactly similar to those obtained for SDS adsorption. The breakthrough volumes were much higher. The $2.1 \mu\text{mol}/\text{m}^2$ CTAB adsorption with the 0.0003 M solution corresponds to 0.00022 moles of CTAB. This amount was contained in 2.2 liters of mobile phase. The experiment duration was two days at $1 \text{ mL}/\text{min}$.

The three apolar phases show L-type concave adsorption isotherms. The two polar phases show S-type convex isotherms [12]. The notable difference is the CTAB adsorption on bare silica. This adsorption is a proof that hydrophobic interaction is not the only driving force for CTAB adsorption on the porous silica surface. Silanol-quaternary ammonium interactions are likely. They induce a cooperative adsorption of more CTAB molecules by hydrophobic interaction.

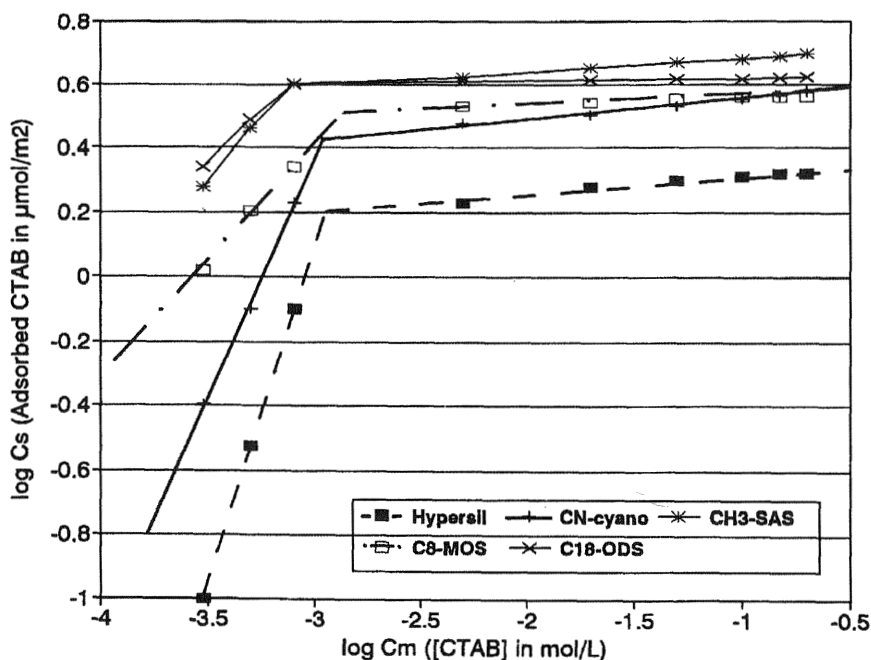


Figure 4.5 Stationary phase versus mobile phase CTAB concentrations in log-log coordinate showing a clear break for the cmc value ($\text{cmc} = 0.0009 \text{ M}$, $\log \text{cmc} = -3.05$).

Micellar Mobile Phases. Figure 4.4B shows the 30°C adsorption isotherm of CTAB on the 0-0.2 M concentration range [11]. The five isotherms are of the H-type reaching a plateau for concentration higher than the cmc [12]. Figure 4.5 shows the corresponding $\log C_s$ versus $\log C_m$ Freundlich plots. The curves are clearly made by two lines. The slope change at the cmc concentration ($\log \text{cmc} = -3.05$) is apparent. For the MOS and ODS phase, an adsorption saturation is observed for CTAB concentration higher than 2 cmc. For the three other phases, a slight increase ($\sim 10\%$) in the CTAB adsorbed amount is noted (Figure 4.4B). This increase is much lower than what was observed with the SDS surfactant (Figure 4.2B).

c) Nonionic Surfactant

Hinze studied the adsorption of nonionic surfactants on a C18 bonded phase (Resolve C18, Table 4.1) [16]. H-type isotherms similar to the ones obtained with ionic surfactants (Figures 4.2 and 4.4) were established for two polyoxyethylene dodecyl ether surfactants (Brij® 22 and Brij® 35). The surfactant adsorption increased beyond the surfactant cmc. The adsorbed Brij® 22 amount increased from $1.4 \mu\text{mol}/\text{m}^2$ at 20 cmc (0.0002 M, $\text{cmc}=10^{-5}$ M) to $2 \mu\text{mol}/\text{m}^2$ at 1700 cmc (0.16 M or 100 g/L), a 40% increase. The increase of the Brij® 35 adsorbed amount was almost continuous. It was about $0.3 \mu\text{mol}/\text{m}^2$ at 2 cmc (0.0002 M, $\text{cmc}=10^{-4}$ M) and $0.9 \mu\text{mol}/\text{m}^2$ at 850 cmc (0.08 M or 100 g/L), a 200% increase. The $\log C_s$ versus $\log C_m$ Freundlich plot of the Brij® 22 adsorption data showed a bilinear curve similar to the plots found in Figures 4.3 and 4.5. The $\log C_s$ versus $\log C_m$ Freundlich plot of the Brij® 35 adsorption data was a straight line without a break at the cmc concentration. Brij® 35 is a polar and highly water soluble surfactant, it may have a low affinity for the apolar bonded Resolve C18 stationary phase [16].

III.3. The Surfactant Adsorbed Layer

a) Physicochemical Structure of the Ionic Surfactant Layer

The structure of SDS and CTAB surfactant layer adsorbed on porous silica was studied by NMR. The C18 (Microsorb ODS, 5 μm , 6 nm pore diameter) and C8 (Microsorb) bonded phases were studied first [17]. The cyanopropyl bonded phase (Microsorb), was also studied [18]. Cross polarization (CP), magic angle spinning (MAS) with high power proton

decoupling were the NMR techniques used to study the surfactant-bonding moiety interactions. Differences between the contact times CP/MAS were found between SDS and CTAB adsorbed molecules. These differences were linked to polarity differences found with surfactant covered phases.

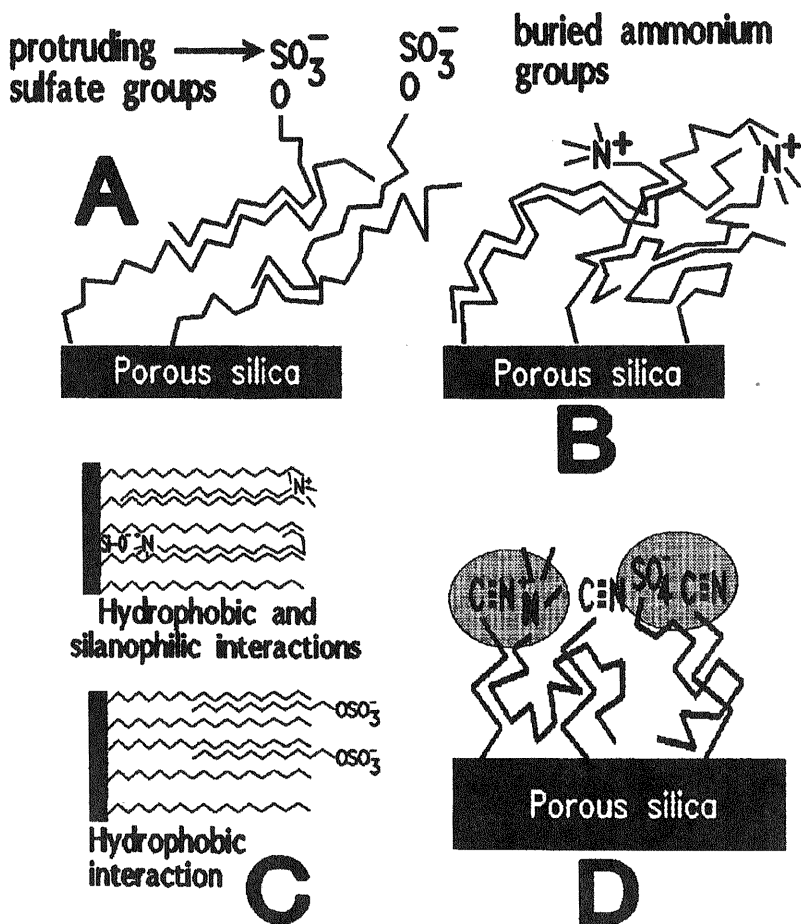


Figure 4.6 Surfactant adsorption on monomeric C18 bonded silica phases. A) the ionic sulfate groups protrude outside the bonded layer making it negatively charged; B) the cationic ammonium groups are buried inside the C18 layer; C) Silanophilic interactions are another way to explain the lower ionic charge observed in case of CTAB adsorption; D) the cyano groups strongly bind to the charged surfactant head either positive or negative. A, B and D from RMN studies [17, 18]. C from chromatographic studies [19].

On alkyl-bonded phases, the RMN results seem to indicate that the trimethyl ammonium polar head of the CTAB surfactant is located inside the thickness of the bonded layer (Figure 4.6A) when the sulfate polar group of SDS protrudes outside as illustrated by Figure 4.6B [17]. This would explain why the SDS covered alkyl-phases are more polar with a negative charge than the CTAB covered phases. These results are coherent with Berthod's work [19]. Berthod thought that the SDS adsorption could occur through hydrophobic adsorption as seen by RMN (Figure 4.6). However, there are two ways to describe the adsorption of CTAB: (i) hydrophobic and (ii) silanophilic adsorption (Figure 4.6C) [19]. The silanophilic adsorption is the interaction between the Lewis acid silanol groups and the basic cationic ammonium groups. It is the only way to explain the CTAB adsorption on the bare silica (Figure 4.4).

The RMN studies showed that there was a strong interaction between the surfactant charged heads and the cyano groups bonded on the Microsorb phase. The charged heads of the adsorbed surfactant molecules are "tied up" by the cyano functionality of the bonded phase (Figure 4.6D) [18]. The negative SDS charge is almost canceled and so is the positive CTAB charge. The stationary phase has just a slightly higher polarity after surfactant adsorption. The surfactant-cyano groups' strong interaction was difficult to disrupt: part of CTAB or SDS was irreversibly adsorbed on Microsorb [18]. Such irreversible adsorption was not observed with the Hypersil® phases [11, 19].

b) Change of the Silica Surface

It was shown that the adsorption of surfactant molecules on the silica surface changes its polarity. Obviously, it also changes its structure, surface area, pore volume and pore size. It was shown that, with SDS and Brij® 35, the surface area lost beyond the cmc was close to 0.6 m^2 per adsorbed surfactant μmole [16]. For example, $0.5 \mu\text{mol/m}^2$ of Brij® 35 were adsorbed on Resolve C18 when the aqueous phase concentration was 0.02 M [16]. The initial surface area of this phase was $180 \text{ m}^2/\text{g}$. The total amount of adsorbed Brij® 35 at 0.02 M is $90 \mu\text{moles}$. The surface area decline is close to $90 \times 0.6 = 54 \text{ m}^2$. The surface area of the stationary phase covered by the Brij® 35 layer at 0.02 M is only $126 \text{ m}^2/\text{g}$, a 30% loss. The surface area loss was only 0.3 m^2 per adsorbed μmole of Brij® 22.

The surface area decrease may be due to distortion of the pore shape or to pore filling with surfactant. The pore structure of the Resolve C18 phase was studied by nitrogen porosimetry [16]. It was found that, for nonionic Brij® 22 and Brij® 35 and for anionic SDS, the general pore shape of the Resolve C18 phase was retained. The pore volume was decreased by surfactant adsorption. These observations suggested that the surfactant molecules were coating the interior walls of the pores without completely filling them [16]. The thick surfactant film formed on the top of the organic bonded layer is responsible for a part of the loss of efficiency observed in MLC with purely aqueous phases.

III.4. Effect of Mobile Phase Additives on Surfactant Adsorption

a) Buffers and Salts

Ionic compounds are frequently added to micellar mobile phases for pH or ionic strength adjustments. Chapter 2 described the significant changes that any salt addition produces on the physicochemical structure of ionic surfactant micelles (mainly: decrease of cmc and increase of the aggregation number). The slope changes in the $\log C_s$ versus $\log C_m$ Freundlich plot for adsorption data obtained with CTAB and SDS mobile phases with 0.1 M NaCl added salts corresponded to the surfactant cmcs in such solutions (0.0002 M and 0.0014 M, respectively, compared to 0.0008 M and 0.0082 M in pure water) [20]. The amount of adsorbed surfactant was found to depend on the stationary phase [11, 16, 19]. A “salting-out” effect was observed with the apolar alkyl phases. In most cases, it increased the amount of adsorbed surfactant by lowering the electrostatic repulsions and enhancing the hydrophobic interactions [20]. Table 4.2 lists the effect of NaCl addition on the adsorption of various surfactants on different LC phases. The addition of NaCl increases the surfactant adsorption by 15 to 40%. The exception, 5 $\mu\text{mol}/\text{m}^2$ of SDS on Hypersil® SAS C1 phase dropping to 4.4 $\mu\text{mol}/\text{m}^2$ when 0.1 M NaCl is added (Table 4.2), was explained by silanol ion-exchange phenomena that are enhanced by NaCl on this phase [20]. The Si-O⁻ ionized groups have no affinity for the anionic SDS.

Table 4.2 Effect of NaCl Additions on the Surfactant Adsorption on Alkyl Bonded HPLC Packings.

Surfactant + NaCl	Stat. Phase	Adsorbed amount $\mu\text{mol}/\text{m}^2$	$t_{1/2}$ h.	Ref.
SDS 0.2 M	Hypersil SAS C1	5.0		[19]
SDS 0.2 M +NaCl 0.1 M -id-		4.4		[20]
SDS 0.2 M	Hypersil ODS C18	4.7		[19]
SDS 0.2 M +NaCl 0.1 M -id-		5.2		[20]
CTAB 0.1 M	Hypersil SAS C1	4.7		[19]
CTAB 0.1 M +NaCl 0.1 M -id-		5.5		[20]
CTAB 0.1 M	Hypersil ODS C18	4.2		[19]
CTAB 0.1 M +NaCl 0.1 M -id-		4.6		[20]
C_{16} Pyridinium Chloride 0.001 M	Davisil C18	3.2		[21]
C_{16} PyrCl +0.01 M NaCl -id-		3.8	1.5	[21]
C_{16} PyrCl +0.1 M NaCl -id-		5.0	7	[21]
C_{16} PyrCl +0.5 M NaCl -id-		5.0	33	[21]
$\text{C}_{16}\text{E}_3\text{PyrCl}$ 0.001 M	-id-	3.4		[21]
$\text{C}_{16}\text{E}_3\text{PyrCl}$ +0.01 M NaCl -id-		3.6	14	[21]
$\text{C}_{16}\text{E}_3\text{PyrCl}$ +0.1 M NaCl -id-		3.8	60	[21]
$\text{C}_{16}\text{E}_3\text{PyrCl}$ +0.5 M NaCl -id-		3.6	150	[21]
$\text{C}_{16}\text{E}_5\text{PyrCl}$ 0.001 M	-id-	2.3		[21]
$\text{C}_{16}\text{E}_5\text{PyrCl}$ +0.01 M NaCl -id-		2.6	15	[21]
$\text{C}_{16}\text{E}_5\text{PyrCl}$ +0.1 M NaCl -id-		2.8	17	[21]
$\text{C}_{16}\text{E}_5\text{PyrCl}$ +0.5 M NaCl -id-		3.1	170	[21]
$\text{C}_{16}\text{E}_8\text{PyrCl}$ 0.001 M	-id-	1.8		[21]
$\text{C}_{16}\text{E}_8\text{PyrCl}$ +0.01 M NaCl -id-		2.1	17	[21]
$\text{C}_{16}\text{E}_8\text{PyrCl}$ +0.1 M NaCl -id-		2.5	57	[21]
$\text{C}_{16}\text{E}_8\text{PyrCl}$ +0.5 M NaCl -id-		2.6	130	[21]

C16 = hexadecyl, E = oxyethylene unit, Pyr = Pyridinium ion. Davisil C18: spherical ODS bonded phase from Alltech, $175 \text{ m}^2/\text{g}$, $t_{1/2}$ = time in minutes necessary to desorb 50% of the adsorbed amount of surfactant at 1 mL/min with the surfactant free NaCl solution (5 cm x 4.6 mm columns) [21].

Table 4.2 presents the results obtained with UV absorbing pyridinium chloride surfactants [21]. The authors studied the “salting out” effect that increased the adsorbed amount on an ODS stationary phase. They also studied the desorption rate of the four ethoxy pyridinium chloride surfactants by passing pure NaCl solutions on the surfactant-covered phase. An exponential decrease of the surfactant loading was obtained. The half time, $t_{1/2}$, to desorb 50% of the surfactant, was between 1.5 hours and 170 hours (a week) [21]. NaCl dramatically decreased the surfactant desorption rate. This is a logical consequence of the “salting out” effect.

b) Organic Modifiers

The first studies of surfactant adsorption isotherms were done with hydro-organic mobile phases in ion-pairing chromatography. The CTAB coverage of unbonded Hypersil® was found to be $1.2 \mu\text{mol}/\text{m}^2$ with 50-50% v-v methanol-water mobile phase containing 0.014 M of CTAB pairing ion [22]. This value is 30% lower than the value obtained with the same phase in pure water ($1.7 \mu\text{mol}/\text{m}^2$, Figure 4.4). Knox obtained even lower values on a Hypersil® SAS phase ($0.2 \mu\text{mol}/\text{m}^2$ with a 30-70% v-v propanol-water phase containing 0.055 M of CTAB) [8]. He was the first to establish surfactant adsorption isotherms [9]. The adsorbed amount of SDS on Hypersil® ODS was as high as $8 \mu\text{mol}/\text{m}^2$ with a 20-80% v-v methanol-water mobile phase containing 0.07 M sodium salts and 0.004 M SDS. Saturation of the stationary phase by CTAB was observed on a RadialPack C18 phase when 60-40% v-v methanol-water (with 0.1 M NaBr and 0.02 M borate buffer) mobile phases containing a CTAB concentration passing the cmc value (0.019 M) were used [23].

It was established that organic modifiers decreased the amount of adsorbed surfactant by competing for adsorption sites and/or by decreasing the hydrophobic interactions [24]. For ionic surfactants, the ability of an organic modifier for surfactant desorption was related to its hydrophobicity. Figure 4.7 shows that the amount of adsorbed surfactant decreases linearly with the organic modifier concentration. The slope of the lines is related to the surfactant desorption strength. Pentanol strongly desorbs ionic surfactants, methanol is less efficient [24].

Physicochemical methods such as contact angle, surface tension measurements and Fourier transformed infrared (FT-IR) spectroscopy were

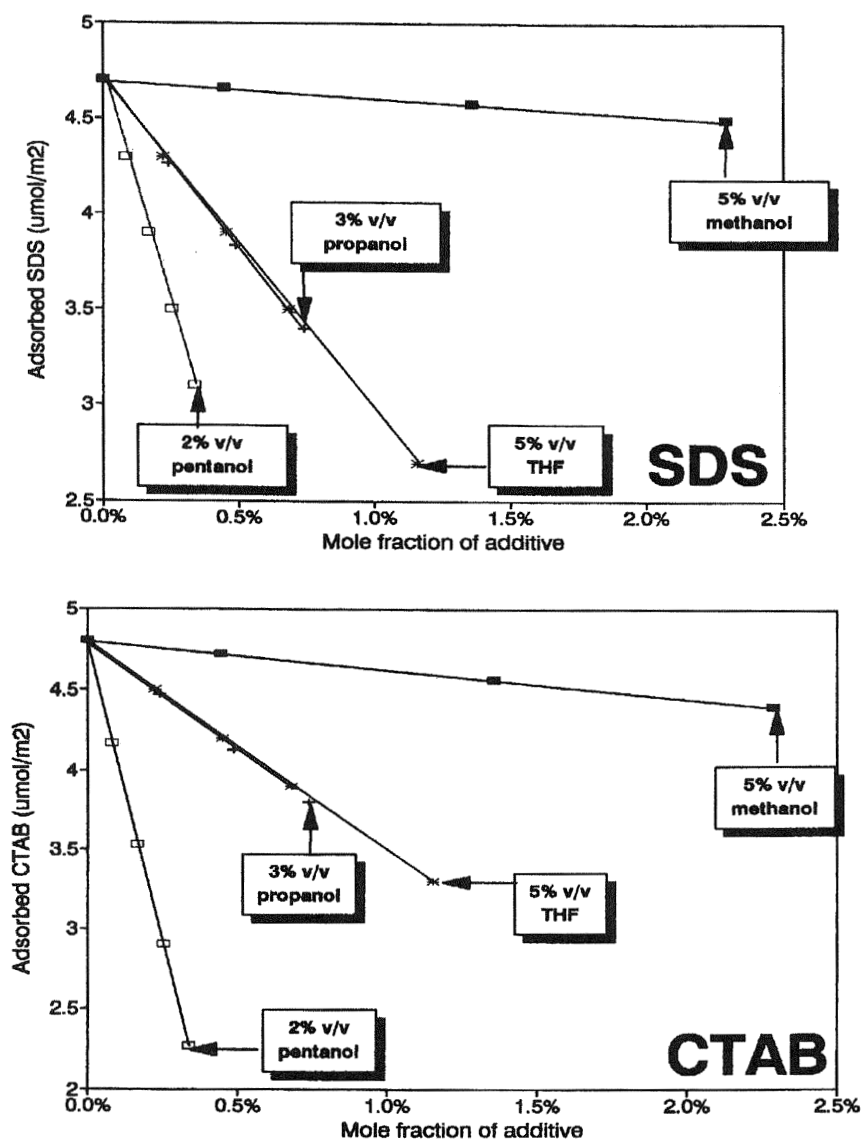


Figure 4.7 Effect of organic additives on the plateau of concentration of adsorbed surfactant. Stationary phase: Hypersil ODS; mobile phase concentration, SDS = 0.2 M, CTAB = 0.1 M, data from [24].

used to investigate the structure of the alcohol-silica bonded layer [25]. It was found that the alkyl chains of propanol and longer n-alcohols interpenetrate the C18 bonded alkyl chains to form a single monolayer similar to the one formed by the alkyl chains of the surfactant molecules (Figure 4.6). The hydroxyl group is oriented toward the aqueous phase. Competition between alcohol and surfactant molecules for adsorption explains the decreased amount of adsorbed surfactant with increasing concentration of alcohol in the mobile phase (Figure 4.7).

The organic additives of the micellar mobile phase affect the surfactant-adsorbed layer. This changes the chromatographic selectivity and efficiency obtained for a set of analytes with the same column. Selectivity and efficiency are studied in other parts of this book.

IV. STATIONARY PHASES AND SELECTIVITY

It was shown that surfactants adsorb at any interface, especially the stationary phase surface. The question is: once a column is well equilibrated with a micellar phase, are we working with a given bonded stationary phase or with a surfactant stationary phase? In other words, does the surfactant coating of the stationary phases render them all similar?

IV.1. *The Role of the Stationary Phase*

To answer to the introduction question, a study of the retention behavior of a set of solutes of various polarities was done with different stationary phases and identical micellar mobile phases [26]. SDS and CTAB micellar phases were used with five different Hypersil® phases. The retention of the polar, apolar as well as ionic solutes studied obeyed Armstrong's three phase model (eq. 3.4, Chapter 3). Table 4.3 lists the P_{WM} solute-micelle partition coefficient and the P_{WS} solute-stationary phase affinity coefficient for different solutes on four bonded phases. The P_{WM} values are similar within experimental errors (see Chapter 5). The P_{WS} values are similar for the C8 and C18 bonded phases. These two phases do behave similarly in classical RPLC. The more polar C1 and CN bonded phases do show significantly lower affinity coefficients. The surfactant layer is there: cationic solutes are not retained by the four bonded phases and hydro-organic mobile phases. Their high affinity for the stationary phases with SDS mobile phases is due

Table 4.3 Solute-Micelle Partition Coefficients, P_{WM} , and Solute-stationary Phase Affinity, P_{WS} , for Four Different Hypersil Bonded Phases.

Solute	Stationary phase				Average value
	SAS C1	MOS C8	ODS C18	CPS CN	
P_{WM}	solute-micelle partition coefficient				
SDS mobile phase					
Toluene	240	290	240	170	235
Caffeine	40	37	38	36	38
Octylbenzenesulfonate	160	300	220	110	200
Benzyltrimethylammon.	1700	2000	1900	500	1500
CTAB mobile phase					
Toluene	280	360	390	190	300
Caffeine	39	23	35	34	33
Benzoic acid	1900	3200	2800	2000	2500
Cetylpyridinium chloride	3000	4700	2900	4300	3700
P_{WS}	solute-stationary phase affinity				
SDS mobile phase					
Toluene	19	150	140	23	-
Caffeine	12	10	5.6	3.5	-
Octylbenzenesulfonate	9.4	35	33	10	-
Benzyltrimethylammon.	420	1400	1500	51	-
CTAB mobile phase					
Toluene	55	190	190	63	-
Caffeine	3.6	1.8	2.3	2.0	-
Benzoic acid	870	760	550	490	-
Cetylpyridinium chloride	850	1000	610	910	-

Data from Ref. 28. Toluene = apolar solute, caffeine = polar solute, sodium octylbenzenesulfonate and benzoic acid = anionic solutes, benzyltrimethylammonium bromide and cetylpyridinium chloride = cationic solutes. Hypersil stationary phase data are listed in Table 4.1.

to the anionic SDS adsorbed layer. It is clear that (i) the solute-micelle partition coefficient does not depend on the stationary phase, (ii) in spite of the surfactant coverage, the polar nature of the bonded stationary phase is maintained [26].

An original study was done with a perfluorinated stationary phase whose properties were compared to a classical C18 bonded phase [27, 28]. An important decrease of the methylene selectivity for a homologue series of alkylphenones was obtained with the fluorinated phase with both an SDS [28] and a C14TAB micellar phase [27]. The decrease in methylene selectivity with fluorinated stationary phases was observed with classical hydro-organic mobile phases [29]. The $\text{CF}_2\cdots\text{CH}_2$ interaction (perfluorinated bonded phases) is much weaker than the $\text{CH}_2\cdots\text{CH}_2$ interaction (alkyl bonded phases). The surfactant adsorbed layer does not change this methylene selectivity. With the C14TAB cationic micellar mobile phase, the selectivity of the fluorinated phase was very different from the one of the C18 phase: alkylphenols were more retained on the C18 phase, polar solutes (e.g., 4-hydroxypropylphenol) were more retained on the perfluorooctyl phase [28]. Similar results were obtained with hydro-organic mobile phases [29]. The layer of surfactant adsorbed on the stationary phase does not completely hinder the solute-bonded moiety interactions.

IV.2. The Effect of the Surfactant Layer

The adsorbed surfactant layer affects the surface of the stationary phase (i) by possibly changing its charge density, (ii) by modifying the stationary phase-mobile phase interfacial tension.

a) Ionic Surfactants and Surface Charge

A layer of ionic surfactant molecules adsorbed on a stationary phase creates a charge density at the silica surface as illustrated in Figure 4.6. The retention of solutes of the opposite charge is dramatically increased as shown by the P_{WS} values of ionic solutes listed in Table 4.3. Organic and inorganic ions were separated by an ion-exchange mechanism with the adsorbed ionic surfactant molecules [30, 31]. A portion of Chapter 13 describes the possible uses of the ion-exchange capability of ionic surfactant covered stationary phases.

b) Interfacial Tension

It was shown that the surfactant adsorption decreased the interfacial tension between the stationary and mobile phases. In the absence of micelles (ion-pair chromatography), the nonionic solute retention factor was affected by this interfacial tension change according to eq. 4.7 [32]:

$$k = k_0 [1 + C/G]^\xi \quad (4.7)$$

where k_0 is the retention factor in the absence of surfactant, C is the mobile phase surfactant concentration ($< \text{cmc}$), G is the Gibbs free energy of adsorption of the surfactant at infinite dilution and ξ is a constant related to the change in interfacial tension caused by the added surfactant. A negative ξ value produces a decrease of the solute retention factor with an increase of surfactant concentration [33].

c) Rapid Micellar Gradient Capabilities

The surfactant adsorption isotherms show an almost horizontal plateau for surfactant concentration above the cmc (Figures 4.3 and 4.5). This property was used to perform rapid analyses with a micelle concentration gradient [34]. In a micellar solution a change in total surfactant concentration serves only to change the concentration of the micelles. If the surfactant-adsorbed layer depends only on the free surfactant concentration, then the stationary phase is not altered by changes in the concentration of the micelles. Therefore it is possible with micellar concentration gradients to speed the elution of strongly retained solutes without affecting the stationary phase [34]. This allows a step gradient back to the initial conditions without re-equilibration time [13]. This rapid gradient capability is not universal in MLC. An almost horizontal plateau in the surfactant isotherm adsorption is required. Significant increases of surfactant adsorption above the cmc were observed especially with nonionic surfactant [16] and/or with polar stationary phases [19]. In such cases, some time (=several column volumes) will be necessary to re-equilibrate the column after a micellar gradient analysis.

V. EQUILIBRATION AND CARE OF THE COLUMN

V.1. *Column Preparation*

It is always recommended to use the same column with the same type of surfactant. A column should be dedicated to the anionic surfactants, a second one to the cationic surfactants, etc. The reproducibility of the results in MLC depends on the column equilibration. The adsorbed layer of surfactant should be done correctly. It was shown that the time to reach the equilibrium between the stationary phase and the mobile phase could be very long in ion-pair chromatography with sub-micellar mobile phases. Two days at 1 mL/min were necessary to equilibrate a 15 cm $\times$ 4.6 mm i.d. column of Hypersil® ODS with a mobile phase containing 0.0003 M CTAB [19]. These low surfactant concentration solutions do not contain micelles. So, they are not used in MLC. With a micellar phase, the equilibration time is reduced. It is possible to use the rapid gradient capability just mentioned above. Typically, a mobile phase containing a high surfactant concentration (10 to 100 cmc) can be used to quickly saturate the column with surfactant. Then 5 to 10 column volumes are used to rinse the column with the mobile phase containing the desired amount of surfactant.

V.2. *Surfactant Desorption*

The recommendation to use the same column with the same surfactant was done because, in some instances, it may be impossible to fully remove the surfactant-adsorbed layer. It was not possible to eliminate a small part of SDS adsorbed on a 1980 Hypersil® ODS phase even when using a pure methanol mobile phase [8]. However, a complete SDS desorption from a 1988 Hypersil® ODS phase was possible with a pure methanol mobile phase [24]. Part of the nonionic Brij 35 surfactant could not be removed from a Resolve C18 phase after a 24-hour elution of water-acetonitrile 70-30% v-v [16]. Conversely, there are many examples where the surfactant layer can be completely stripped off the stationary phase [11, 19, 21, 24]. It was suggested that partial irreversible surfactant adsorption was due to a tight insertion of the surfactant alkyl chains in the alkyl moieties of the bonded layer of densely grafted phases [35]. A column could be used with different

surfactants (anionic and cationic) after a complete “stripping” with pure methanol [24].

Most surfactants are very soluble in pure methanol. This mobile phase is recommended for surfactant desorption and column cleaning. A methanol-propanol 75-25% v-v may be necessary in rare occasions. 20 to 50 column volumes is the minimum quantity of methanol to elute and to remove the adsorbed surfactant. An experimental test should be used to ensure a complete surfactant desorption. The adsorbed layer of surfactant decreases the solute mass-transfer between the stationary phase and the mobile phase. This produces band-broadening. A dramatic efficiency decrease is observed [16, 24, 35]. The experimental test can be described as follows: A test mixture is prepared to measure the efficiency of an RPLC new column with a classical hydro-organic mobile phase. For example, a 50 $\mu\text{mol/L}$ benzophenone + biphenyl solution was used in Ref. 26 with an ODS stationary phase and a methanol-water 75-25% v-v mobile phase. The retention parameters of the test solutes (retention factors and peak efficiencies) are measured with the hydro-organic mobile phase and the column before any surfactant exposition. These parameters are similarly checked after the column has been exposed to surfactants, rinsed with pure methanol and re-equilibrated with the reference hydro-organic mobile phase [24]. If the retention factors and the peak efficiencies of the test solutes are similar to their original values, a complete recovery of the initial stationary phase surface can be assumed. A small decrease of the column efficiency may be due to column aging.

V.3. Column Care

Most micellar solutions used in MLC contain more than 90% v/v water. These aqueous solutions are able to dissolve minute amounts of silica especially at elevated temperature ($T > 30^\circ\text{C}$) and/or pH values higher than 6. Silica dissolution is deadly for the column. It should be reduced to a minimum. This problem is solved by saturating the aqueous mobile phase in silica. This is done, without causing any band broadening, by placing a saturating precolumn just after the pump and before the injection valve. This precolumn can be a short (5 cm x 4.6 mm i.d.) column packed with a

10 μm bare silica phase to reduce the pressure build-up. Since the precolumn also acts as a filter, it should be checked regularly.

A micellar solution should not stay in contact with the bonded silica-based stationary-phases when the chromatographic system is not used. The first reason is that the chromatographic hardware does not like salt solutions such as ionic surfactant solutions. Corrosion may occur in unattended systems containing ionic surfactant solutions. Most surfactants are solids that are weighted to prepare the micellar solutions. In a nonworking system, evaporation can produce crystals' formation around the pump plungers and seals. Such crystals may obstruct the system producing capillary or frit clogging. They may also scratch the pump piston. The second reason is that the static water-rich micellar solution may produce silica dissolution and/or Si-C bond ruptures that can ruin the column. A very essential rule of care is: *a micellar phase should never stay motionless in a chromatographic system.*

A cleaning procedure will be indicated. It may be tedious to clean the system every evening and to re-equilibrate it every morning. We found that a micellar phase does not harm a chromatographic system as long as it is not static. This means that it is possible to keep a micellar phase overnight if the pump is not turned off. If the operator knows he will go on working with the same micellar phase and he wants to leave, he can reduce the flow rate to a minimum value (often 0.1 mL/min) to reduce the pressure and pump wear. Then he should place the detector outlet Teflon tubing back in the mobile phase reservoir to recycle the micellar phase. This procedure ensures that the system will not run dry (unless a leak occurs) overnight. It also guarantees the good equilibrium of the adsorbed surfactant in the column. Last, it saves the stationary phase since the recycled mobile phase is saturated in dissolved silica and will not dissolve more of it.

If the MLC system will not be used for the weekend or several days, it should be cleaned according to the following three step procedure.

- First, the micellar phase is replaced by 100% pure water. The pump is flushed rapidly and the system, valve, column, detector and tubing, is rinsed with 10 to 20 column volumes of pure water. A higher flow rate can be used if the pressure does not increase too much. The pure water viscosity is only 1 cP.

- Next, a 100% methanol mobile phase is used to remove the adsorbed surfactant in the chromatographic system. A *low flow rate* (0.5 mL/min or less) should be used at the beginning because the methanol-water mixture has a high viscosity which is significantly increased by the desorbed surfactant. Once the pressure decreases, the flow rate may be resumed. At least 10 column volumes of pure methanol should be passed through the column.
- Then, the hydro-organic mobile phase used to test the column efficiency can be loaded in the system (5-10 column volumes). The test itself will be done when the system will be restarted. The power can be turned off. The column contains a classical hydro-organic phase without ions that will not damage it.

The complete procedure takes about half an hour. It should not be neglected. The 100% water step should not be bypassed. A brutal change from a buffered micellar mobile phase to a 100% methanol phase may produce salt crystallization. The column and even the pumping system could be damaged. The last step, with a hydro-organic mobile phase, can be bypassed. But it is good practice to check for column performances once every week.

If the two rules, a low flow rate with the micellar phase during the night and the 30 min cleaning procedure for the weekend, are respected, the lifetime of the columns used in MLC are comparable and even superior to what is usual in RPLC. These rules were followed with a chromatographic system using a 15-cm ODS column. The column performances were maintained for more than two years of intensive MLC use.

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Partitioning Behavior in Micellar RPLC Systems

I. INTRODUCTION

In Micellar Liquid Chromatography (MLC), the mobile phase consists of an aqueous solution of surfactant at a concentration above the critical micellar concentration (cmc), in contact with an alkyl-bonded stationary phase. MLC is a Reversed-Phase Liquid Chromatography (RPLC) mode, with micelle acting as a mobile phase modifier. It is also often necessary to add a small concentration of an organic solvent to improve the efficiency and better control the elution strength and selectivity of the mobile phase. The technique has also been named as pseudo-phase liquid chromatography. This term was coined to describe separations where significant partitioning of a solute occurs to discrete aggregates dissolved in the mobile phase, rather than to bulk solvent.

Micellar mobile phases of anionic, cationic, nonionic and zwitterionic surfactants are used in conjunction with different bonded stationary phases (including C8, C18 and cyano). Considerably less surfactant (usually <0.2 M) is used compared to the organic modifier content in an analogous traditional separation. A variation in the concentration of surfactant is translated into an increase in the concentration of micelles in the solution, whereas the number of monomers of surfactant remain constant. As a consequence, the characteristics of the stationary phase modified by the adsorption of surfactant are very stable, and usually, a regular retention behavior is observed as a function of the concentration of surfactant.

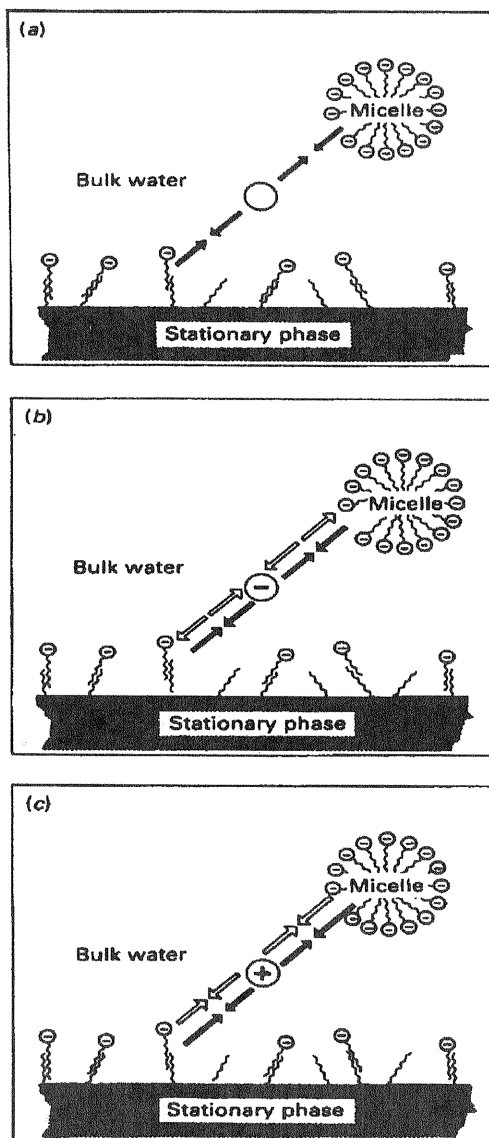


Figure 5.1 Solute-micelle and solute-stationary phase hydrophobic ($\rightarrow$) and electrostatic interactions ($\rightleftharpoons$) with an anionic surfactant: (a) nonpolar solute, (b) anionic solute and (c) cationic solute.

Micellar systems share the basic components of an RPLC system, that is, a nonpolar stationary phase and a polar aqueous mobile phase. However, conventional RPLC with aqueous-organic mobile phases are homogeneous, whereas micellar solutions are microscopically heterogeneous, being composed of two distinct media: the amphiphilic micellar aggregates (micellar pseudophase) and the surrounding bulk aqueous or aqueous-organic solvent that contains surfactant monomers in a concentration approximately equal to the cmc. In micellar solutions, the solutes are preferentially solubilized into or onto the micellar assembly, a process which is dynamic. The solutes localized in micelles experience a microenvironment that is dramatically different from that of bulk solvent in terms of polarity and fluidity. This is reflected by micelle-induced perturbations of physico-chemical properties of the solutes, including changes in solubility, acidity, photophysical properties and reaction rates.

The complexity of MLC is much greater than that of conventional RPLC with aqueous-organic solvents, because of the number of possible interactions with both mobile and stationary phases (Fig. 5.1). The solutes in the mobile phase can interact electrostatically with the charged outer-layer of ionic micelles, and hydrophobically with their lipophilic interior. The steric factor can also be important. The modification of the stationary phase by adsorption of surfactant monomers, which creates a "micelle-like" surface, gives rise to similar interactions with the solutes. The combination of these interactions cannot be duplicated by any traditional pure or mixed solvent system. While micellar solutions will never totally replace traditional aqueous-organic eluents, they offer several interesting alternatives to separation work.

The basic mechanism of separation in MLC is fairly well understood and there is a reasonable theoretical foundation on which to build. MLC is a fascinating example of the use of a secondary chemical equilibrium in liquid chromatography. The primary equilibrium is the partitioning of the solute between bulk mobile phase and stationary phase, and the secondary equilibrium is the partitioning to micelles. This secondary equilibrium is affected by a great variety of parameters: type and concentrations of surfactant and additives such as salts or organic modifiers (for instance, alcohols), and pH. The current knowledge on MLC interactions is exposed

in this chapter. The implications of such interactions on the elution strength and selectivity of micellar eluents are considered in Chapter 7.

II. THE THREE-PHASE MODEL

II.1. Solute-Micelle and Solute-Stationary Phase Interactions

Armstrong and Nome [1] proposed a three-phase (stationary phase, bulk aqueous solvent and micellar pseudo-phase) model, to explain the chromatographic behavior in an RPLC system of a solute eluted with a mobile phase containing a surfactant above the cmc. Such a treatment has allowed a theoretical description of MLC, and greater understanding and utilization of this chromatographic technique.

According to the three-phase model, the retention of a solute is controlled by three competing reversible equilibria, namely partitioning (or binding) from bulk aqueous solvent to micelles, partitioning from bulk aqueous solvent to the alkyl-bonded stationary phase, and direct transfer from micelles to the stationary phase. The first equilibrium takes place inside the mobile phase, and the latter equilibrium can be neglected in most situations, but it is significant for highly nonpolar (water-insoluble) solutes, which have a great affinity for both stationary phase and micelles. The partitioning equilibria are described by three coefficients: P_{WM} (between water and micelles), P_{WS} (between water and stationary phase), and P_{MS} (between micelles and stationary phase). It is the first partition coefficient, P_{WM} , that imparts uniqueness to MLC. The coefficients P_{WS} and P_{WM} have opposing effects on the retention of solutes: as P_{WS} increases the retention increases, whereas as P_{WM} increases the retention is reduced due to increased partitioning into micelles.

The retention behavior of solutes will depend on the type of interactions with the micelles and with the surfactant-modified stationary phase. Nonpolar solutes should only be affected by hydrophobic interactions (Fig. 5.1a). For these solutes, different proportions of nonpolar, dipole-dipole and proton donor-acceptor interactions between solutes and

surfactants are expected. But for solutes that are charged, two distinct situations can be considered:

- (i) the sign of the charges on the solute and surfactant are the same (Fig. 5.1b), or
- (ii) the sign of the charges on the solute and surfactant are opposite (Fig. 5.1c).

The first situation is encountered when an anionic solute is eluted with an anionic surfactant, or a cationic solute is eluted with a cationic surfactant [e.g., dissociated phenol with the anionic surfactant sodium dodecyl sulfate (SDS), and protonated benzylamine with the cationic surfactant dodecyl trimethylammonium bromide (DTAB), on a C18 column] [2]. Electrostatic repulsion from the micelle should not affect the retention as the solute will still reside in the bulk solvent phase, and therefore, will move down the column. In contrast, repulsion from the surfactant-modified stationary phase should cause a decreased retention, and the solute may elute in the dead volume. However, it may be retained by hydrophobic interaction with the stationary phase, although this effect will be reduced by the electrostatic repulsion. Because due to different hydrophobic interactions, dissociated phenol and 2-naphthol are well separated with SDS.

The second situation appears when a solute is chromatographed with an oppositely charged surfactant, where electrostatic attraction occurs between both species. Electrostatic attraction between solute and micelle will complement any hydrophobic interaction, and thus, it can be expected that the solute will remain in the mobile phase for a longer period of time, decreasing the retention. However, electrostatic and hydrophobic interactions with the stationary phase are often sufficiently large to offset micellar attraction and thus the retention will increase. That is why dissociated phenol and 2-naphthol are retained to a greater extent with DTAB than with SDS on a C18 column [2].

Nonionizable solutes should only experience hydrophobic interactions. It has been shown, however, that any molecule with a dipole moment can profoundly be affected by electrostatic effects [3].

II.2. Classification of Compounds According to Their Elution Behavior

The function of the micellar pseudo-phase in MLC has been compared to that of the organic modifier in traditional RPLC, as for most solutes an increase in the concentration of surfactant in the mobile phase results in a decreased retention. This is in contrast to reversed-phase ion-interaction chromatography, where the surfactant concentration is below the cmc (*i.e.*, no micelles exist), and the addition of an ionic surfactant will increase the retention of compounds that interact electrostatically with it. However, in MLC, the elution strength increases with micelle concentration only if the solute interacts with micelles.

Armstrong and Stine [4] proposed a classification of compounds into three groups, according to their elution behavior with a micellar mobile phase:

- (i) compounds binding to micelles
- (ii) nonbinding compounds
- (iii) antibinding compounds

Compounds that associate or bind to micelles show decreased retention when the concentration of micelles in the mobile phase is increased. For compounds that do not associate with micelles, the retention may remain unaltered by the micelle contents (nonbinding), or may increase with increasing micelle concentration (antibinding). Fig. 5.2 shows plots of the reverse retention factor ($1/k$) vs. micelle concentration ($[M]$) for each type of behavior. Binding, nonbinding and antibinding compounds give straight-lines of positive, zero and negative slope, respectively. The behavior most frequently observed is binding to micelles, while the antibinding behavior is quite uncommon.

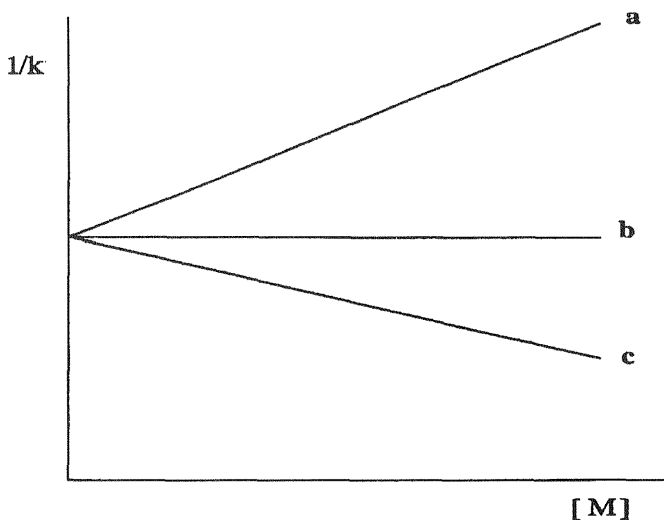


Figure 5.2 Typical plots of MLC retention vs. mobile phase micelle concentration for a: (a) binding solute, (b) nonbinding solute, (c) antibinding solute.

Most antibinding compounds with anionic micelles are negatively charged and those with cationic micelles are positively charged. These types of compounds have also been observed with micelles composed of amphoteric surfactants. In contrast, antibinding has never been observed between a charged solute and oppositely charged micelles. It is thus apparent that electrostatic repulsion is an important factor in antibinding behavior. However, there are also many positively charged compounds that bind to cationic micelles and negatively charged compounds that bind to anionic micelles, which is favored through hydrophobic interactions. Consequently, lines of zero slope in Fig. 5.2 indicate either that there are no attractive or repulsive forces between the solute and micelles or, more likely, that these forces are approximately equal and negate one another. It should be said that it is sometimes difficult to predict the exact retention behavior of an organic ion.

The antibinding behavior cannot be observed with stationary phases that adsorb an appreciable amount of surfactant (*i.e.*, C8 or C18 bonded phases). For these phases, when the column acquires the same charge as the micelles, and no hydrophobic interaction occurs, similarly charged solutes tend to elute in the dead volume of the column. In contrast, when using stationary phases which do not adsorb large quantities of surfactant (*i.e.*, C1 or preferably cyano-bonded phases), one can observe increased retention when eluting with higher a concentration of micelles in the mobile phase.

Antibinding results from a compound being strongly excluded or repelled from the micelle. In fact, the solute is not only excluded from the micelle, but also from the double-layer around the micelle. The solute is thus forced onto the stationary phase. A negative water-to-micelle partition coefficient ($P_{WM} < 0$) has been assigned to these compounds, which has in principle no physical meaning. However, as compounds that bind to micelles each have a characteristic coefficient, compounds that are excluded from micelles may have a characteristic negative parameter. This parameter may be useful, since in some cases it can be correlated to the degree of electrostatic repulsion between solute and micelles.

There are a variety of methods to measure partition coefficients of solutes to micelles. Much less attention is given to solutes that do not seem to interact with micelles or, at least, do not follow models that require a specific type or amount of interaction. The repulsion of a solute from a micelle is sometimes implied (for instance, in the micellar inhibition of a reaction), but rarely treated in a rigorous quantitative manner. Part of the reason for this is the lack of experimental techniques that can measure zero or negative interactions. In addition, experimental phenomena that can be explained by the binding of solutes to micelles are often thought to be more relevant and interesting. Using MLC one can detect and measure positive (*i.e.*, binding), negative (*i.e.*, antibinding) or zero (*i.e.*, nonbinding) interactions between solutes and micelles. Furthermore, one can monitor a solute's change in behavior (from binding to nonbinding to antibinding or *vice versa*), when a small change in the environment and/or micelles occurs.

II.3. Description of the Retention Behavior

In MLC, the retention factor is related to the concentration of monomers of surfactant in the form of micelles (*i.e.*, total concentration of surfactant minus cmc), through very simple equations. Three theoretical approaches, based on the three-phase model, have been proposed to formulate the retention of binding solutes for liquid chromatographic systems at various micellar concentrations: the partitioning model of Armstrong and Nome [1], and the equilibrium approaches of Arunyanart and Cline-Love [5], and Foley [6]. Jandera and Fischer [7] extended the mathematical treatment to antibinding solutes. The equations derived are similar and allow the evaluation of the strength of solute-micelle and solute-stationary phase interactions. Also, another pseudo-phase can be substituted for the micelle (such as a cyclodextrin or crown ether), without appreciably changing the theoretical equations.

a) Equation of Armstrong and Nome

Armstrong and Nome [1] extended to micellar media the classic model of Martin and Synge, and Herries et al. [8]. The model describes the situation that a solute experiences in an ideal system formed of a number of identical plates, where partitioning equilibria take place. The transitions that can occur in the three environments that exist in a micellar chromatographic system (*i.e.*, the aqueous phase, the micelles and the stationary phase) were considered (Fig. 5.1). The mass fraction of solute in each environment and each theoretical plate was obtained from P_{WM} and P_{WS} . The P_{MS} coefficient was not included in the model, since it can be obtained by combination of the two former ($P_{MS} = P_{WS}/P_{WM}$).

Based on the expression giving the maximally occupied theoretical plate, the following equation was obtained:

$$\frac{V_e - V_o}{V_s} = \frac{k}{\phi} = \frac{P_{WS}}{1 + v(P_{WM} - 1)[M]} \quad (5.1)$$

where V_e represents the total volume of eluent needed to elute a given solute from the column, V_s is the volume of the active surface of the stationary phase, V_o the column dead volume, $\phi = V_s / V_o$ the phase ratio, and v the partial specific volume of monomers of surfactant in the micelle. The calculation of P_{WM} requires knowledge of v (e.g., 0.246 L/mol for SDS, 0.364 L/mol for cetyltrimethylammonium bromide, CTAB, and 1.084 L/mol for Brij® 35).

When micelles are not present in the mobile phase, eq. 5.1 is reduced to the partitioning equation of conventional RPLC:

$$V_e = V_o + V_s P_{ws} \quad (5.2)$$

b) Equation of Arunyanart and Cline-Love

Arunyanart and Cline-Love [5] rewrote the pseudo-phase retention equation in a similar form. They used retention factors and binding constants, rather than the corresponding elution volumes and partition coefficients. The following three chemical equilibria were considered:

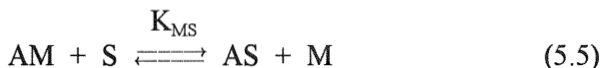
(i) association of the solute in bulk water, A, with the stationary phase binding sites, S:



(ii) association or binding of the solute in bulk water with a monomer of surfactant in the micelle:



(iii) direct transfer of the solute from the micelle to the stationary phase:



The third equilibrium was also neglected in this approach. Substitution of the equilibrium constants K_{WS} and K_{AM} in the expression giving the retention factor leads to:

$$k = \phi \frac{[AS]}{[A] + [AM]} = \frac{\phi K_{WS} [S]}{1 + K_{AM} [M]} \quad (5.6)$$

Since $[S]$ is usually constant, this quantity can be included in the partition constant K_{WS} . The constant K_{AM} is referred to the association of the solute with a surfactant monomer in the micelle, and should be multiplied by the aggregation number to find the constant referred to the whole micelle.

c) Equation of Foley

The assumptions made in the equilibrium model proposed by Foley [6] are not very different from those of the previous authors [1, 5]. The association between the solute in bulk water and the micelles (eq. 5.4) is considered a secondary equilibrium, which affects the retention of the solute in the absence of micelles, given by k_0 .

$$k = \frac{1}{1 + K_{AM} [M]} k_0 \quad (5.7)$$

This equation is again similar to the equation proposed by Armstrong and Nome (eq. 5.1). The retention factor of free solute, k_0 , coincides with ϕP_{WS} in eq. 5.1 and $\phi K_{WS} [S]$ in eq. 5.6, whereas K_{AM} corresponds to the same constant in eq. 5.6, and to the product $v(P_{WM} - 1)$ in eq. 5.1 when the volume of aqueous phase is taken as total volume of mobile phase. Also, the basic pseudo-phase equation of retention (eq. 5.1) can be algebraically rearranged in at least 12 different related forms [9]. Although obtaining one form from another is mathematically trivial, there are certain statistical and other advantages in using different variations.

d) *Partition Coefficients*

The three models given by eqs. 5.1, 5.6 and 5.7 lead to similar linear equations $1/k$ vs. $[M]$, and can be rewritten as:

$$k = \frac{K_{AS}}{1 + K_{AM}[M]} \quad (5.8)$$

or

$$\frac{1}{k} = \frac{1}{K_{AS}} + \frac{K_{AM}}{K_{AS}} [M] \quad (5.9)$$

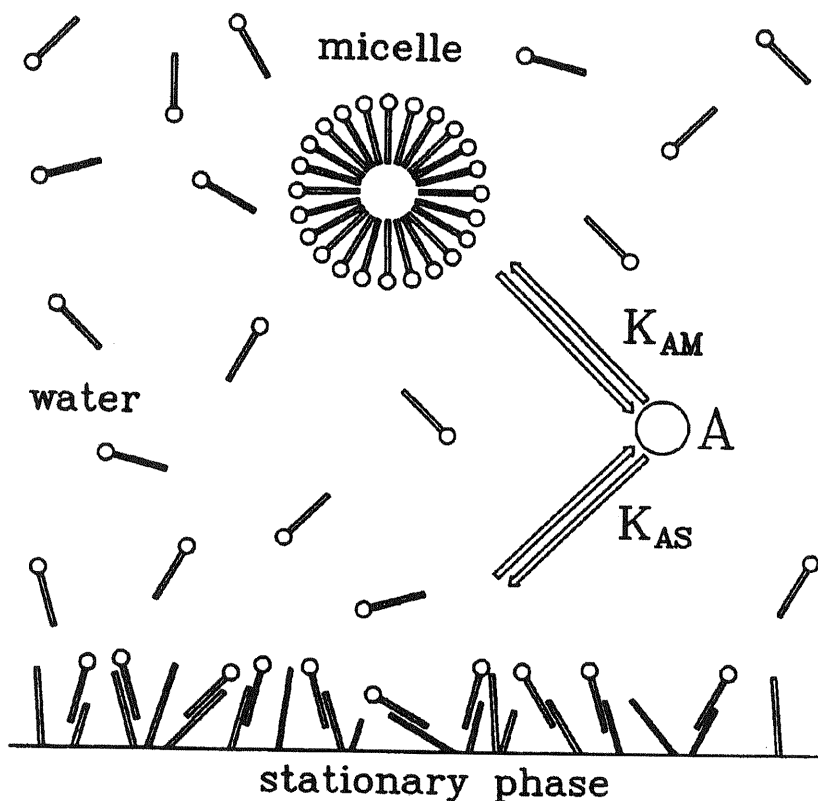


Figure 5.3 Solute-micelle, K_{AM} , and solute-stationary phase, K_{AS} , binding constants in micellar mobile phases.

Figure 5.3 illustrates the three-phase model with indication of the partition constants K_{AM} and K_{AS} . The values of K_{AM} and K_{AS} give information about the solute affinity to micelles and to the stationary phase, respectively.

One consequence of the partitioning treatment is that the measured values of K_{AS} , K_{AM} and K_{MS} for a compound using the same micellar mobile phase, but two different stationary phases, will result in different values of K_{AS} and K_{MS} , but identical values of K_{AM} . By the same token, the measured values of a compound on a single stationary phase, but with two different micellar mobile phases, will result in different values of K_{AM} and K_{MS} but identical values of K_{AS} .

The constant K_{AS} should indicate the different degree of adsorption of surfactant. This was not however observed in a study performed with several solutes and CTAB as surfactant [10]. The high values of K_{AS} obtained with cyano-silica, and the unexpectedly low values with C18-silica, clearly showed that the adsorption of surfactant was not the only factor to take into account. The subjacent stationary phase (the bonded moiety) probably still plays a role in the interaction of the solute between bulk water and stationary phase. In spite of the surfactant coverage, the polar nature of the bonded stationary phase is maintained.

e) Jandera and Fischer Approach

Equations 5.8 and 5.9 describe the retention of solutes that can form associates, or inclusion complexes, both with micelles and surfactant adsorbed on the stationary phase. This is the case for neutral compounds and compounds with an opposite charge to the surfactant. However, as indicated above, compounds having the same charge as the surfactant will be excluded from the micelles and repelled by the modified stationary phase, unless other interactions exist that neutralize the electrostatic repulsion. For solutes repelled from the micelles, the retention increases with the concentration of surfactant, which supposes an apparent negative value for K_{AM} . Since this constant is the ratio of the equilibrium concentrations of solute between bulk water and micelles, it should be positive.

Jandera and Fischer [7] pointed out to the severe inconsistency of the equations of retention stated above, to describe the behavior of antibinding solutes. They postulated that as the repulsion forces hinder the molecules of a solute to come into close contact with the molecules of surfactant, a part of the stationary phase with adsorbed surfactant becomes inaccessible. This means that only a fraction of the real volume of stationary phase, V_{So} , affects the phase ratio in the column. The relative reduction in accessible volume, ΔV_S , is proportional to the amount of adsorbed surfactant, Q_{cmc} , which is assumed to be constant above the cmc:

$$\frac{\Delta V_S}{V_{So}} = f_S Q_{cmc} \quad (5.10)$$

Similarly, a fraction of the volume of mobile phase could not participate in the interactions with the solute:

$$\frac{\Delta V_m}{V_{mo}} = f_m [M] \quad (5.11)$$

where f_S and f_m are the fractions of stationary and mobile phases inaccessible to the solute, respectively, which depend on both solute and surfactant.

According to this model, four equations were formulated corresponding to different situations. The first coincides with eq. 5.8, which is valid for solutes that are not excluded from both stationary phase and micelles. For solutes repelled by the adsorbed monomers of surfactant on the stationary phase, but associated with the micelles:

$$k = \frac{K_{AS}}{1 + K_{AM} [M]} (1 - f_S Q_{cmc}) \quad (5.12)$$

whereas for solutes repelled by both the stationary phase and micelles:

$$k = K_{AS} \frac{1 - f_s Q_{cmc}}{1 - f_m [M]} \quad (5.13)$$

A similar equation can be obtained for solutes nonexcluded from the stationary phase but repelled by the micelles ($f_s = 0$ in eq. 5.13). The two latter situations correspond to antibinding solutes. It is not likely, however, that a solute is repulsed from the stationary phase by the adsorbed surfactant and at the same time is associated with the surfactant in the mobile phase, or *vice versa*, owing to the inherently similar nature of the interaction forces between the molecules in the two phases.

Finally, all the equations describing the retention in MLC can be formulated as:

$$\frac{1}{k} = c_0 + c_1 [M] \quad (5.14)$$

where the coefficients c_0 and c_1 will have a different sign according to the nature of the interactions in the stationary phase and micelles.

f) Deviations from the Partitioning Model

Equations 5.1, 5.6 and 5.7 predict decreased retention with increased micelle concentration, if the solute partitions to the micelle. A plot of retention vs. total amount of surfactant, over a wide range of surfactant concentration, will show that below the cmc there will be little change in retention, unless there are ion interaction effects. On the other hand, at very high surfactant concentration, the solute can elute near the dead volume and further increases in concentration will not lower the retention. Experimental points taken near this region might deviate from theory and should be avoided.

The equations of retention have been verified experimentally for a large number of solutes (nonpolar, polar, neutral and ionic), surfactants

(anionic, cationic, nonionic and zwitterionic), and column materials (C8, C18 and cyano). They are also valid for mobile phases containing a constant amount of organic modifier. However, it should be noted that certain assumptions have been made to formulate the equations, regarding the micellar and chromatographic system. It was assumed that:

- (i) the cmc and aggregation number of micelles are not affected by the small amounts of solute being chromatographed,
- (ii) binding of the surfactant to the stationary phase does not appreciably affect the retention time of the solute or, if the retention is affected, this binding reaches a level of saturation at or before the cmc of the surfactant is reached,
- (iii) an increasing concentration of micelles does not alter their aggregation number or geometry,
- (iv) the stoichiometry of the solute-micelle "complex" is 1:1.

In cases where these assumptions are not valid, deviations from the expected behavior can be found. In fact, the three latter assumptions are only reasonable at a limited range of low surfactant concentration. For instance, adsorption of SDS does not occur on naked silica until 0.1 M concentration is reached. Therefore, the model should fail with silica as the stationary phase. In fact, it was observed that the change on the silica surface due to SDS adsorption caused a variation in the K_{AS} values of several nonionic and ionic solutes, so that the plots of $1/k$ vs. SDS micellar concentration were no longer linear [10].

At high concentration, some surfactants such as CTAB, undergo "sphere to rod" transitions. Also, upward curving plots will occur with 1:2 stoichiometry or greater. Apparently, owing to these reasons, nonlinearity was observed in the $1/k$ vs. total SDS concentration plots for some amino acids and peptides (Fig. 5.4) [11]. Two different lines were obtained for

these compounds, depending on the examined range (0.02-0.08 M and 0.1-0.2 M SDS).

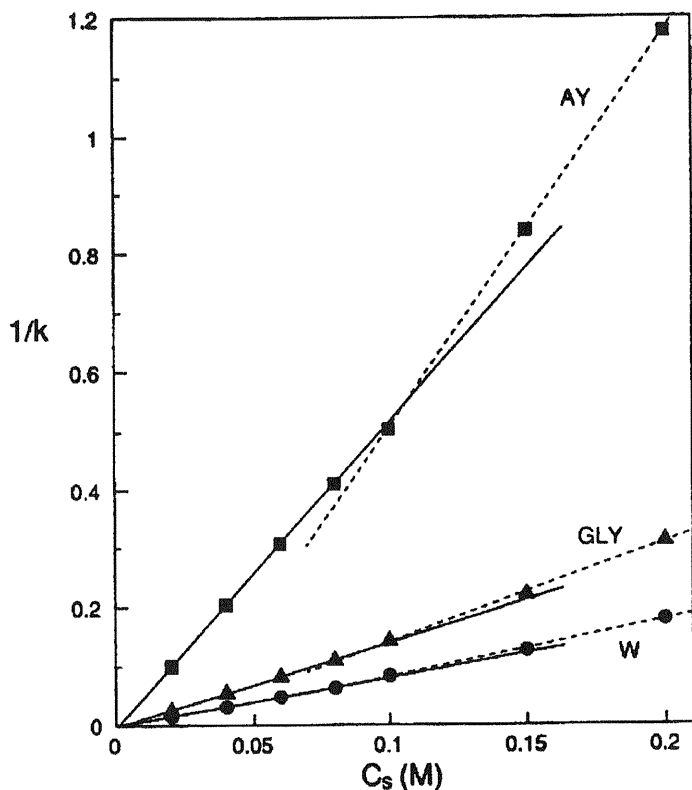


Figure 5.4 Plot of $1/k'$ vs. SDS concentration for ■ alanyl-tyrosine (AY), ▲ glycyl-leucyl-tyrosine (GLY), and ● tryptophan (W), eluted at pH 2.5 using two different ranges of surfactant concentration: Solid lines, 0.02-0.08 M SDS; dashed lines, 0.1-0.2 M SDS. Reprinted from Ref. 11 with permission of Elsevier.

Finally, deviations from the model are observed for highly and low retained solutes. In the former case, irreversible adsorption of the solute on the stationary phase may occur, whereas in the latter, the elution of the solute will always take place in the dead volume. The high errors obtained for hydrophobic compounds may arise, at least partially, from using large

concentrations of surfactant which are needed to elute these compounds from a reversed-phase column, in a reasonable time.

III. HYBRID MICELLAR MOBILE PHASES

III.1. Use of Organic Solvents as Modifiers in Micellar Solutions

a) Enhancement of Elution Strength

One of the most serious problems of pure micellar eluents is their weak elution strength, often lower than the elution strength of methanol-water mobile phases. Shorter retention times can be achieved by using higher surfactant concentrations and shorter chain-length bonded stationary phases. The elution strength can also be increased by the addition of an organic solvent. The term hybrid was proposed for ternary eluents of water-organic solvent-micelles, and will be used here [12].

The addition of small percentages of 1-propanol to micellar mobile phases was first recommended by Dorsey et al. [13], to enhance the chromatographic efficiency and decrease the asymmetry of chromatographic peaks. Since then, several organic solvents have been studied as modifiers in MLC. Of these, short and medium chain alcohols (*i.e.*, methanol, ethanol, propanol and butanol) have shown to be the most suitable. Less frequent has been the use of pentanol [14, 15]. Only a few reports have appeared on the MLC behavior of solutes in the presence of other organic solvents commonly employed in conventional RPLC, such as acetonitrile [13, 16, 17], and tetrahydrofuran [18, 19]. Micellar mobile phases allow the use of organic solvents in aqueous solution at molar concentrations well above their normal solubility limit in water alone. For example, the water solubility of pentanol is ca. 0.30 M, whereas in 0.285 M SDS micellar medium, it increases to ca. 0.94 M [17].

A study was made on the effect of different organic additives (*i.e.*, alcohols, alkane diols, alkanes, alkylnitriles, and dipolar aprotic solvents such as dimethyl sulfoxide and dioxane) upon the elution strength of micellar

SDS, hexadecyltrimethylammonium chloride (CTAC), and Brij® 35 mobile phases, for two neutral test solutes, benzene and 2-ethylanthraquinone, retained on a C18 stationary phase [17]. The test solutes were chosen due to the fact that benzene is relatively water soluble and 2-ethylanthraquinone is virtually water insoluble. Thus, they represent two extremes in hydrophobicity. The results indicated that the presence of alcohols, diols, alkylnitriles, and dipolar aprotic solvents, in the micellar mobile phases, resulted in a diminution of the retention factors for the two test solutes. In contrast, the presence of the alkane additives (*i.e.*, pentane, hexane, cyclohexane) did not greatly alter the retention.

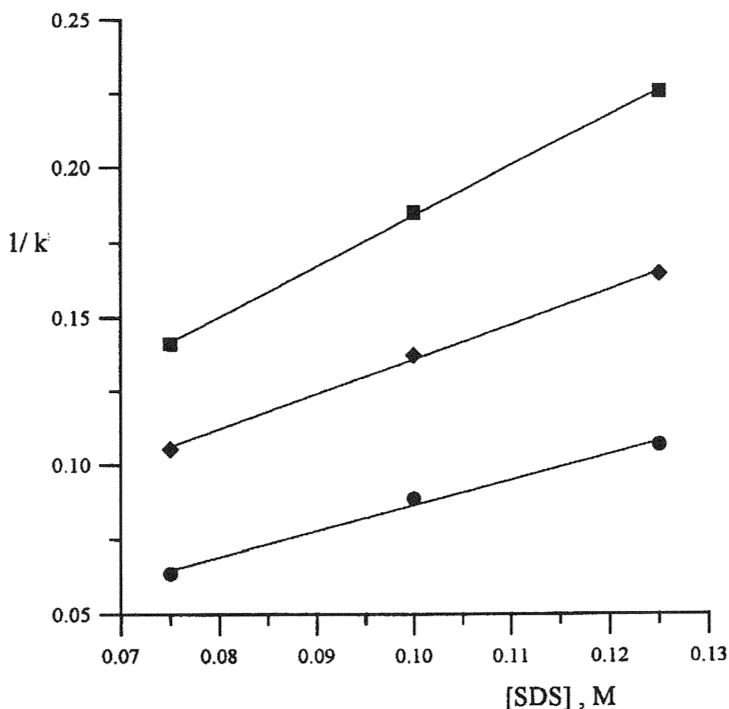


Figure 5.5 Effect of the hydrophobicity of alcohols on the retention of amiloride, eluted with SDS mobile phases containing 3% 1-propanol (●), 3% 1-butanol (◆), and 3% 1-pentanol (■).

The use of alcohols as additives in MLC can result in dramatic reductions in the elution times of solutes, compared to those observed with pure aqueous micellar mobile phases. The retention factors decrease as the carbon number (hydrophobicity) of the alcohol is increased (Fig. 5.5), and the reduction is more pronounced for micellar solutions containing greater amounts of the additive (Fig. 5.6). The organic modifier effects upon the retention however are attenuated as the surfactant concentration is increased. On the other hand, the effects are greater for more hydrophobic solutes. Thus, the reduction in retention factors observed upon the addition of organic modifiers to micellar mobile phases depends, not only upon the identity and concentration of the organic modifier, but also upon the concentration of surfactant and the hydrophobicity of the test solute. Relatively low amounts of organic modifier can greatly affect solute retention in MLC. This is particularly important when attempting to separate very hydrophobic components.

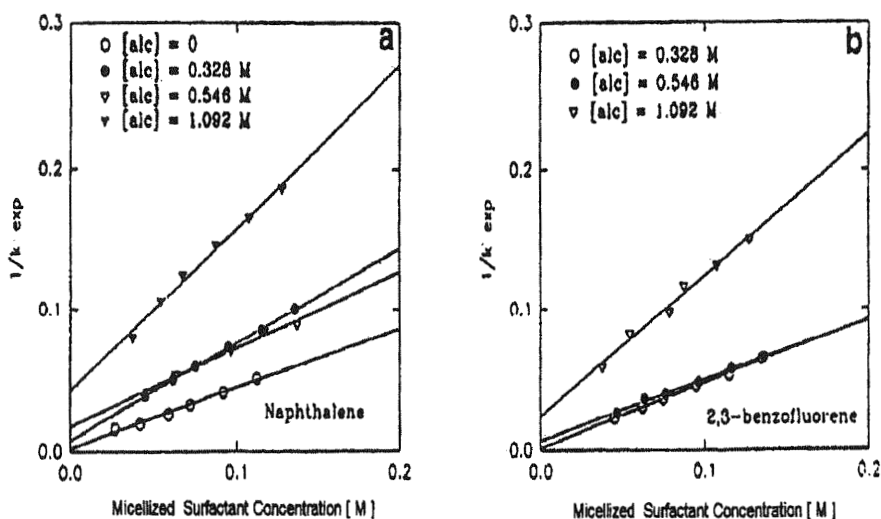


Figure 5.6 Effect of the concentration of alcohol on the retention of: (a) naphthalene, and (b) 2,3-benzofluorene, in SDS mobile phases modified with 1-butanol. Reprinted from Ref. 20 with permission of Elsevier.

The elution strength of the alcohol is usually greater than that of the surfactant. However, for positively charged solutes such as catecholamines in acidic medium, anionic surfactants can originate an important reduction in the retention due to the high affinity of the solutes towards the negatively charged micelles [21].

b) Displacement of Partitioning Equilibria

The relative effectiveness of an organic modifier in MLC appears to be directly related to its own ability to partition and bind to the micellar pseudo-phase. That is, the better the organic modifier binds to the micellar assembly, the greater its ability to alter the retention of solutes. Eq. 5.9 is also valid for hybrid micellar eluents (Fig. 5.5). For these eluents, solute binding constants to micelles and the partitioning into the stationary phase both decrease as a result of the addition of the modifier, especially for highly hydrophobic solutes. However, the K_{AM}/K_{AS} ratio increases and, therefore, the elution power of the mobile phase is greater.

The addition of alcohols to micellar mobile phases causes changes in certain micellar properties, such as the aggregation number and the cmc of the surfactant. The observed reductions in retention in hybrid systems are however too large to be rationalized in terms of these changes. The reductions should be explained by the modification of the micro-environment of the micelles and the stationary phase.

The organic additive effects upon MLC retention may be due to the modification of the micelle produced by the introduction of molecules of modifier in its palissade, and to the change in the nature of the surfactant-modified stationary phase. The addition of organic modifier also increases the affinity of the solute for the bulk aqueous solvent in the mobile phase, which becomes more nonpolar. This significantly alters the equilibrium of the solute away from the micelle and stationary phase towards the bulk aqueous phase which becomes more nonpolar.

III.2. A Micellar or an Aqueous-Organic Mobile Phase?

The concentration of organic solvent added to the micellar mobile phase should not be very high, since this might reduce the role of micelles and

bring the system closer to an aqueous-organic system. Large concentrations of modifier can totally disrupt the micelle structure, as the hydrophobic effect, the main driving force for micelle formation, is reduced. The maximum allowable concentration depends on the type of modifier and surfactant, and is usually not known. As a rule of thumb, the volume percentage of organic solvent should be kept below 15-20%. Some separations reported in the literature were carried out with hybrid mobile phases containing too large amounts of modifier, the role and integrity of micelles were thus not entirely clear [22-24].

When hybrid micellar eluents were first used, they were severely criticized. It has been argued that when organic modifiers are used, this type of chromatography loses some of its appeal [25]. However, most reported procedures in MLC, since 1985, utilized these eluents. The experience acquired in our laboratories on the development of analytical procedures for the determination of different drugs (*e.g.*, β -blockers, diuretics, narcotics, steroids, stimulants and sulfonamides) has shown that, in most instances, the retention of solutes with pure micellar eluents is excessive, which forces one to add a modifier to achieve adequate retention times.

It may seem logical to assume that the separation mechanism with aqueous surfactant-organic solvent mobile phase is similar to that with conventional aqueous-organic solvent, rather to pure aqueous surfactant mobile phase, since the effect of adding an organic solvent is to sacrifice the role and effectiveness of micelles. However, as long as the integrity of micelles is maintained, addition of a modifier to a micellar mobile phase will not create an aqueous-organic system, even though in hybrid systems the interactions between solutes and micelles are reduced, and the stationary phase is more similar to that of a conventional aqueous-organic system.

In effect, organic solvents modify the structure and composition of the stationary phase, as they solvate the hydrocarbonaceous bonded phase. Scott and Simpson [26] studied the adsorption isotherms of different organic modifiers on RPLC phases and showed that over 90% of the C18 phase was covered with 1-propanol at a concentration of 3% (w/v). Therefore, with a micellar mobile phase that includes a small concentration of organic solvent, the stationary phase resembles a solvated phase in an aqueous-organic system, rather than in a pure aqueous micellar mobile phase.

The chromatographic behavior of alkyl homologous series is useful for the investigation of retention. In aqueous-organic systems, the retention of a homologous series is related to the number of carbon atoms, for each solute in the series, through the following relationship [27]:

$$\log k = \log \alpha(\text{CH}_2) n_c + \log \beta \quad (5.15)$$

where n_c is the number of carbon atoms in the homolog, $\alpha(\text{CH}_2)$ is the nonspecific selectivity of a methylene group, and β is the contribution to the retention from the functional group common to the series. This regular increase in retention due to the addition of a methylene group is recognized as a measure of hydrophobic interaction in a given RPLC system.

In contrast to this behavior, several authors have noted that a linear relationship between k and n_c seems to exist in MLC, when either a pure aqueous micellar or a hybrid micellar mobile phase is used [28, 29]. This has been observed for SDS, CTAB and Brij® 35 as micelle-forming surfactants with C8 and C18 stationary phases, and *n*-alkylbenzenes, 2-alkylanthraquinones and *n*-alkylphenones as homologous series. It has been suggested that a different retention mechanism exists for homologous series in an aqueous surfactant-organic solvent mobile phase with respect to an aqueous-organic solvent system, and that pure aqueous micellar and hybrid micellar mobile phases are similar. One practical advantage to be gained from the linear k vs. n_c relationship, in MLC, is that a greater number of solutes of the homologous series will be eluted per unit time in the isocratic mode, compared to that of conventional RPLC. This behavior is further commented in Chapter 9.

III.3. Mobile Phases Containing Surfactants and High Concentration of Modifiers

Li and Fritz [30] studied the effect of some surfactants [*i.e.*, tetraethylammonium bromide (THPA), CTAB, SDS, dioctylsulfosuccinate,

polyoxyethylene(4)dodecyl ether (Brij® 30), polyoxyethylene(20)sorbitan monostearate (Tween-60), polyoxyethylene-polyoxypropylene copolymer (Pluronic® L-31), 1-dodecanol and 1,2-decanediol], in conventional acetonitrile-water and methanol-water mobile phases with C18 columns. Under the working conditions, the formation of micelles was unlikely. Actually, THPA never forms micelles even in pure water because it has a symmetrical tetrahedral geometry. However, the presence of surfactants as additives in aqueous-organic mixtures greatly improved the separation of several groups of compounds: alkylbenzenes, polycyclic aromatic hydrocarbons (PAHs), alkylphenols, and some other aromatic compounds. Compared to separations obtained without the surfactants, shorter retention times and sharper peaks were obtained. The retention times of late-eluting compounds were reduced by a larger percentage than the retention of earlier peaks. This effect is similar to that obtained with gradient elution but in this case isocratic elution with an aqueous-organic eluent, containing an appropriate surfactant, was used.

This type of chromatography can be considered a bridge between MLC and conventional RPLC, because surfactants were used at concentrations above their cmc in aqueous solution, but without micelle formation. The observed additive effect could arise in two ways. First, the surfactant molecules might be adsorbed on the surface of the stationary phase, with the long alkyl or polyoxypropylene chains interacting with the C18 chains and the hydrophilic head groups sticking out. This would give a more hydrophilic surface and thus reduce the retention times of solutes. Second, hydrophobic interactions might be established between the solute molecules and the long alkyl or polyoxypropylene chains of the discrete surfactant molecules, in the mobile phase. Hydrogen bonding may play a role when both solute and surfactant contain potential hydrogen bond formation centers, as in alkylphenol separations with Brij® 30 or Tween-60. In the experimental conditions of these separations, no adsorption of surfactant molecules could occur on the surface of the stationary phase, the second mechanism was thus probably correct.

Increasing concentrations of surfactant in the aqueous-organic solvent eluents resulted in progressively lower retention factors. Interestingly, eq. 5.9 was followed for these mobile phases, however, instead of the concentration of micelles, the concentration of total surfactant was

used. The treatment assumed a 1:1 association between solute and surfactant, and consequently, the linear range was limited to rather low concentrations of surfactant. On the other hand, a linear relationship was found between $\log k$ of alkylbenzenes and alkylphenols, and the carbon number of the series, for all surfactants studied. This relationship suggests that the retention mechanism of the systems agreed with that found in conventional aqueous-organic RPLC, rather than in MLC.

IV. EVALUATION OF PARTITION COEFFICIENTS

IV.1. Advantages of the MLC Approach

There is a significant amount of experimental support for the three-phase model in MLC. First, plots of retention data fit the theoretical expressions (see Section II.3), for a variety of solutes which bind to micelles. Second, the experimental values of K_{AM} and K_{AS} are independent of the stationary phase and micellar system, respectively. Furthermore, micellar binding constants evaluated by MLC are in good agreement with those obtained by other independent methods (*e.g.*, spectroscopic, potentiometric, kinetics, solubility, etc.) [5, 11, 31]. As an example, Table 5.1 shows the calculated partition coefficients P_{WS} and P_{WM} , and related binding constants, for several phenols, quinols and catechols, obtained from chromatographic elution with SDS, compared to previous data. The agreement is satisfactory for phenols where spectrophotometric methods were used. Slight differences, however, are observed between the chromatographic and kinetic data for quinols and catechols. Appendix III lists the partition coefficients and binding constants that we found preparing this book.

According to eq. 5.9, a linearized plot of $1/k$ vs. $[M]$ yields a slope K_{AM}/K_{AS} (units M^{-1} or L/g) and a dimensionless intercept $1/K_{AS}$. Thus, the binding constant K_{AM} (M^{-1} or L/g) can be calculated as the ratio of the slope to the intercept, and K_{AS} coincides with the reciprocal of the intercept. Remembering that $K_{AM} = v (P_{WM} - 1)$, the calculation of the P_{WM}

Table 5.1 Partition Coefficients (P_{WS} and P_{WM}) and Binding Constants (K_{AM}) for Several Compounds Eluted with Sodium Dodecyl Sulfate Mobile Phases on a C18 Column [31] compared to non-chromatographic methods.

Compound	P_{WS}	P_{WM}	K_{AM} (L/mol) MLC	K_{AM} (L/mol) other methods
Phenol or Benzene-1-ol	9.7	40.2	10.2	8 ^a 9.9 ^a
4-Methylbenzene-1-ol	28.6	95.0	24.4	24 ^a
4-Ethylbenzene-1-ol	75.8	245.0	63.5	65 ^a
4- <i>n</i> -Propylbenzene-1-ol	174.2	450.0	116.7	140 ^a
4- <i>t</i> -Butylbenzene-1-ol	389.0	995.0	258.4	280 ^a
3,5-Dimethylbenzene-1-ol	69.4	229.6	59.5	64 ^a
2,4,5-Trimethylbenzene-1-ol	179.2	513.6	133.3	125 ^a
2,3,5,6-Tetramethylbenzene-1-ol	(460)	(1150)	(300)	200 ^a
Catechol or Benzene-1,2-diol	6.3	27.4	6.9	4 ^b
4-Methylbenzene-1,2-diol	17.6	72.4	18.6	10 ^b
4-Cyanobenzene-1,2-diol	5.6	28.1	7.1	5 ^b
3- <i>i</i> -Propylbenzene-1,2-diol	75.2	225.0	58.3	37 ^b
4- <i>t</i> -Butylbenzene-1,2-diol	156.2	431.0	111.8	75 ^b
Hydroquinone or Benzene-1,4-diol	1.7	14.5	3.5	3 ^b
2-Methylbenzene-1,4-diol	3.0	21.1	5.2	6 ^b
2-Chlorobenzene-1,4-diol	4.7	34.0	8.6	7 ^b
2-Phenylbenzene-1,4-diol	32.4	202.0	52.2	85 ^b
2- <i>t</i> -Butylbenzene-1,4-diol	50.2	244.4	63.3	65 ^b
2,3,5-Trimethylbenzene-1,4-diol	9.4	53.8	13.7	12 ^b

^a Spectrophotometric methods; ^b Kinetic methods, in parenthesis, extrapolated values.

dimensionless water-micelle partition coefficient is trivial if the correct unit for v is used. Reviews have been published on the evaluation of these coefficients in MLC [6, 32]. Unfortunately, the dimension problem was ignored.

Table 5.2 K_{AM} Binding Constants in L/mol for Diverse Neutral Solutes in SDS and CTAB Micellar Systems.

Compound	References										
	[2]	[5]	[10]	[11]	[29]	[31]	[33]	[34]	[35]	[36]	[37]
SDS mobile phases											
Anthracene		5325							5440		
Benzene	18.9	25.8			17.1		19.2	20.3	23.5		
Benzyl alcohol				10.4			8.8			11.8	
Naphthalene		242		235			290	353	245	217	
Nitrobenzene	22.1			23.2			23.1			25.9	
Phenol	9.6					10.2	9.4	9.5		10.5	
Pyrene		8065							8360		
Toluene	52.9	50.0	63.3	54.2	59.3		52.4	56.5		76.1	44.8

Compound	References					
	[7]	[10]	[29]	[33]	[36]	[38]
CTAB mobile phases						
Benzene			23.7	40.2	47.2	35.9
Benzonitrile	17.5			19.4	27.2	17.9
Benzyl alcohol				13.5	17.3	12.8
Chlorobenzene				157	548	104
Nitrobenzene	27.0			36.9	54.7	33.3
Phenol				71.4	79.5	38.5
Toluene		142	129	142	204	83.3

Although binding constants (K_{AM}) are usually reported, it should be noted that the figure depends on the units of the partial specific volume of monomers of surfactant in the micelle (L/mol or L/g). It may be more convenient, therefore, to indicate water-micelle partition coefficients (P_{WM}).

A nonexhaustive literature compilation of these coefficients, as measured by MLC, is given in Appendix III. The binding constants are listed addressing the unit problem. In some cases, the partition coefficients were calculated from the binding constants given in the referenced articles. It can be observed that chemically bonded reversed-phase columns were generally employed, with octadecylsilica and octylsilica stationary phases mostly used. Sometimes cyano, C1 and silica columns were also used. Binding constants have been calculated mainly for SDS and CTAB micellar systems. Nonionic and zwitterionic surfactants have also been studied, although only to a small extent. Most work was done using pure and unbuffered micellar mobile phases at room temperature. When a modifier was introduced in the mobile phase, it consisted of a short or medium chain alcohol, such as methanol, ethanol, propanol or butanol, or a salt such as sodium chloride at low concentration. When pH was fixed, a phosphate buffer was usually used.

Some solute-micelle binding constants for aromatic compounds with SDS and CTAB, obtained by different authors under identical experimental conditions, are included in Table 5.2. Only the chromatographic column could change (octadecylsilica and octylsilica). Good agreement exists when different K_{AM} values are available, especially in the case of SDS micelles, even for very hydrophobic compounds for which the error in these determinations is usually high.

The evaluation of solute-micelle binding constants has important implications outside the field of chromatography, such as micellar catalysis, tertiary oil recovery, and enzyme and membrane modeling. MLC is a powerful technique for the determination of these constants, in comparison to classical methods. The use of MLC in this field has several advantages:

- (i) the evaluation can be made for all the compounds experiencing a chromatographic retention in the system that varies when the concentration of surfactant in the mobile phase is changed. This is quite common and the compounds do not need to experience a change in their spectroscopic characteristics in micellar media, as when spectroscopic methods are used,

- (ii) simultaneous determination of the constants of several solutes is possible, as the retention times of a mixture of the solutes can be measured from a single injection.
- (iii) solute concentration need not be known, and
- (iv) impurities, if present in the sample, are chromatographically separated and will not interfere.

Moreover, binding constants can be obtained from independent sources to predict retention factors. This is useful to facilitate systematic optimization. An example of this type of prediction has been given for perylene which showed a high retention and the intercept of the $1/k$ vs. $[M]$ plot was essentially zero with a C18 column and SDS in the mobile phase [5]. The retention factor at a given micelle concentration and a reported value of the binding constant were taken to estimate the value of the intercept in eq. 5.9, and to further predict the retention at varying micelle concentration. The agreement between experimental and predicted values was excellent.

IV.2. Some Considerations on Partitioning Calculation

a) Critical Micelle Concentration

For any aqueous surfactant solution, there is a relatively small range of concentrations below which virtually all surfactant is present as monomers, and above which virtually all additional surfactant is present in micellar form. This micellization phenomenon causes significant changes in the bulk physical properties of the solution. The cmc is an important parameter which deserves careful attention in order to minimize uncertainties in K_{AM} measurement.

The cmc values for the most widely used surfactants in MLC are well known (e.g., 8.1×10^{-3} M for SDS, 1.3×10^{-3} M for CTAB, and 1.0×10^{-4} M for Brij® 35) [39]. However, in a hybrid micellar mobile phase where an organic solvent is present, the cmc values change and are often not

referenced. Therefore, they should be evaluated prior to the calculation of binding constants.

Different modifiers show different behavior. A progressive decrease in the cmc has been observed in SDS solutions, with respect to the absence of modifier, for all alcohols, methanol excepted [40]. The reduction in the cmc is larger when the length of the alcohol chain is increased, and occurs even for small amounts of alcohol. At moderate concentrations of propanol, butanol and pentanol, the concentration of micelles almost coincides with the total concentration of surfactant. Similarly to methanol, the cmc increases with the concentration of organic solvent for acetonitrile and tetrahydrofuran [40].

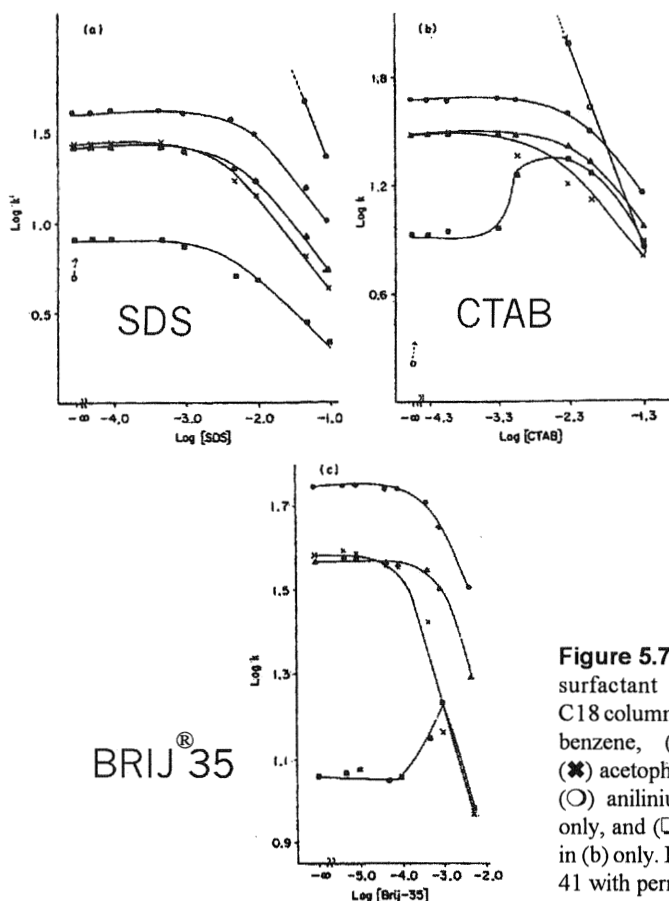


Figure 5.7 $\text{Log } k$ vs. log surfactant concentration for C18 columns. Compounds: (●) benzene, (▲) nitrobenzene, (✕) acetophenone, (■) phenol, (○) anilinium chloride in (a) only, and (□) sodium benzoate in (b) only. Reprinted from Ref. 41 with permission of Elsevier.

The determination of cmc values is made by the measurement of some property of the solution, as a function of surfactant concentration. The cmc is taken as the point of intersection of the extrapolated segments of the measured property at concentrations of surfactant below and above the cmc. One of the properties that has been used to measure cmc values is the retention in a micellar mobile phase of a neutral solute that associates strongly with micelles [41]. Figure 5.7 shows plots of $\log k$ vs. logarithm of surfactant concentration for acetophenone, anilinium chloride, benzene, nitrobenzene, phenol and sodium benzoate, and the three surfactants SDS, CTAB and Brij® 35. The solutions also contained 3% 1-propanol to improve the efficiency of the chromatographic peaks. The dramatic change in retention as micellization occurred is quite obvious. The values of cmc for the three surfactants were calculated from these plots and the results were compared to conductimetric measurements. A relatively good agreement of the chromatographic and literature values (chromatographic method: 4.7×10^{-3} , 3.6×10^{-3} and 3.4×10^{-4} M, and conductimetric method: 7.3×10^{-3} , 1.3×10^{-3} and 1.0×10^{-4} M, for SDS, CTAB and Brij® 35, respectively) was evidence that it is truly solubilization of the solutes by the micelles that causes elution, at relatively high concentration of surfactant.

b) Volume of Stationary Phase

For convenience, the partition coefficients P_{WS} and P_{MS} are usually treated by considering the entire volume of the silica support particles. One should keep in mind, however, that only the surface bonded layer is actually involved in the partitioning equilibria. The calculation of P_{WS} from eq. 5.1 requires knowledge of V_s , which cannot be determined easily. Usually, the difference between the empty column volume and the packed column void volume is taken as V_s , which gives an overestimation of this volume since it includes the entire volume occupied by the silica solid support particles, rather than just the true stationary phase. Although the use of such a value will result in accurate values for P_{WM} , the values obtained for P_{WS} and P_{MS} will be expected to be significantly in error, with underestimated partition coefficients. An approach that completely excludes any volume associated with the base silica material should thus be used.

Borgerding et al. [29] developed an accurate procedure for determining the chromatographic phase ratio. In this procedure, measurement of the total weight of the packing material in the column, and of the cumulative pore volumes of unbonded silica support and bonded C18 packing materials, before and after exposure to the micelle-forming surfactant, is made. In this way, any volume associated with the base silica material is completely excluded, since the stationary phase volume is assumed to be that portion of the silica pore volume filled upon bonding the octadecylsilane (or other alkyl ligand) chains and, in the presence of micelles, by sorption of the surfactant to the C18 chains.

c) Errors in the Slope and Intercept of the Equation of Retention

The evaluation of K_{AM} and K_{AS} is usually made from linear regression of the experimental data of retention factors and micelle concentration, according to eq. 5.9 (remember $K_{AS} = \phi K_{WS}$). It is evident that K_{AM} will be affected by a larger error than K_{AS} . Considering that:

$$K_{AM} = A/B \quad (5.16)$$

where A and B are the slope and intercept of the straight-line, the error will be given by:

$$\Delta K_{AM} = (B \Delta A + A \Delta B)/B^2 \quad (5.17)$$

ΔA and ΔB being the errors in the slope and intercept, respectively. It should be noted that the error in K_{AM} is not only affected by the errors in the slope and intercept, but also by their values.

The intercept of the straight-line decreases (K_{AS} increases) with the hydrophobicity of the compound. High uncertainties in K_{AS} and even higher in K_{AM} , will result for compounds showing very low intercepts (close to zero). These are compounds with an important hydrophobic character,

experiencing a high retention in the MLC system (*e.g.*, anthracene and pyrene). The high uncertainties in the retention factors of hydrophobic compounds often yield negative intercepts, that is, negative values for K_{AS} and K_{AM} . It has been shown that the application of weights to the linear fitting (eq. 5.9) of $(1/k, [M])$ experimental data, or the use of nonlinear regression (eq. 5.8) improves greatly the accuracy of the binding constants [42]. With these data treatments, achievement of negative intercepts is less frequent.

The statistical treatment of $1/k$ vs. $[M]$ plots indicates that the error in the slope tends to increase when working with a compound having either very small or very large binding constants. There are, therefore, certain limits to the applicability of the procedure with a given stationary phase. Consider, for example, the retention of naphthalene with a C18 reversed-phase column [1]. Because of naphthalene's great affinity for the stationary phase and low affinity for water, an appreciable concentration of SDS micelles in the mobile phase was needed to achieve elution in a reasonable time. In fact, it was difficult to obtain a sufficient amount of data at low enough surfactant concentration to calculate an accurate K_{AM} binding constant for naphthalene, using this stationary phase. In contrast, naphthalene did not interact as strongly with a cyano stationary phase. In this case, sufficient data could be collected to calculate the binding constant. However, the slope of the $1/k$ vs. $[M]$ plot was large and the intercept close to zero, thus, small changes in the slope of the line resulted in relatively large changes in the intercept.

On the other hand, the interaction of hydroquinone with a C18 bonded stationary phase was very low. This lack of interaction was even more pronounced with the cyano column [1]. Consequently, the retention times of hydroquinone with a cyano column were not sufficiently different or consistent in the presence or absence of micelles in the mobile phase. In this particular case, it was difficult to collect adequate data for proper treatment.

Limitations such as those described for naphthalene (with the C18 column) and hydroquinone (with the cyano column) would be more serious if one were limited to a single stationary phase. Fortunately, several different polarity bonded stationary phases are available. As a result, a very wide variety of compounds can be chromatographed using micellar mobile phases

Table 5.3 K_{AM} Binding Constants in L/mol for Aromatic Compounds and SDS Mobile Phases with and without Modifiers [36].^a

Compound	No Modifier	10% MeOH	3% PrOH	5% PrOH	10% PrOH	3% BuOH	5% BuOH	10% BuOH
Benzene	23.5	17.4	19.1	15.9	16.9	16.5	13.3	10.9
Benzyl alcohol	11.8	13.0	8.2	7.2	6.5	5.5	3.4	4.8
Benzamide	12.3	13.6	7.6	3.6	4.6	4.6	2.2	4.2
Toluene	76.1	45.1	54.9	54.7	43.9	44.2	38.8	16.5
Benzonitrile	20.4	20.2	14.2	11.1	10.6	9.0	6.4	7.2
Nitrobenzene	26.0	22.0	18.2	15.9	13.9	12.6	7.3	7.7
Phenol	10.5	12.1	9.4	7.6	8.8	6.6	5.9	6.4
2-Phenylethanol	20.8	22.9	14.6	12.0	11.4	19.1	4.8	6.8
Chlorobenzene	105	60.0	72.5	62.0	55.5	50.6	28.4	17.5
Phenylacetonitrile	30.7	34.0	21.1	14.4	14.5	12.4	6.6	7.5
3,5-Dimethylphenol	57.9	66.9	40.2	24.6	32.0	24.7	14.4	12.9
Naphthalene	217	466	227	207	118	96.9	24.0	21.2
1-Naphthol	190	124	79.8	52.0	62.1	44.7	25.3	17.6

^a MeOH = methanol, PrOH = 1-propanol and BuOH = 1-butanol.

Table 5.3 (continued)

Compound	No Modifier	10% MeOH	3% PrOH	5% PrOH	10% PrOH	3% BuOH	5% BuOH	10% BuOH
2-Naphthol	168	146	73.2	60.6	55.4	41.8	29.3	16.3
1-Naphthylamine	248		62.2	44.6	35.3	32.0	18.4	12.9
Pyrene				415		197	67.6	23.0
Phenanthrene			63.3	162		174	75.2	22.2
2,3-Benzofluorene						458	93.2	26.4
Fluorene			1330	530		179	61.8	22.3
Fluoranthene				376		229	77.9	25.0
Acenaphthylene			496	196		129	56.4	22.1
Acenaphthene			381	190		249	71.4	23.1
Anthracene				331		229	91.8	26.0

^a MeOH = methanol, PrOH = 1-propanol and BuOH = 1-butanol, all K_{AM} constants in L/mol.

and treated accordingly. It has been proposed that a stationary phase should be changed for a more polar one to decrease the errors in the evaluation of binding constants for hydrophobic compounds (the order of increasing polarity is the following: C18 $\approx$ C8 < C1 < cyano < silica). This should decrease the partition coefficients between the aqueous phase and stationary phase, and increase the intercept of the straight-line. It was however checked that the reduction in the errors was minor when a C8 column was used instead of a C18 column [32]. The advantage of using a more polar column is still the possibility of eluting the compounds out of the column or reducing their retention times.

If the solubility of a hydrophobic compound in the aqueous phase is increased by the addition of an organic modifier, such as an alcohol, the displacement of the equilibria towards the aqueous phase will enable the calculation of the binding constants. In effect, the addition of alcohol decreases K_{AS} and K_{AM} , and the associated errors. Figure 5.6 shows the increase in the intercept of the straight-line of $1/k$ vs. $[M]$ for hydrophobic solutes, when 1-butanol at different concentrations is added to an aqueous SDS micellar solution.

The K_{AM} binding constants of several benzene derivatives and PAHs, eluted with hybrid SDS micellar mobile phases, are given in Table 5.3 for different alcohols at varying concentration. The decrease in K_{AM} due to the addition of alcohol to the mobile phase was higher for the most hydrophobic compounds. It was also more important with increasing length (decreasing polarity) of the alcohol chain. Finally, K_{AM} decreased with the percentage of alcohol in the mobile phase. It is interesting to note that it was possible to calculate the constants for all compounds in the presence of butanol. This is in contrast to the results obtained when no additive was utilized in the mobile phase.

The addition of an alcohol usually decreased K_{AM} , regardless of the surfactant nature. For aromatic compounds, this has been checked for SDS and CTAB [36, 43]. However, in the presence of cationic CTAB micelles, the binding constants were higher than in the presence of anionic SDS. This can be explained by the favorable electrostatic interactions between the positively charged CTAB head groups and the unlocated charge of the

aromatic ring of the solutes. In fact, the difference in K_{AM} values with CTAB, relative to SDS, was greater for naphthalene derivatives.

V. THE SOLUBILITY LIMIT THEORY

The approximately zero intercepts obtained for hydrophobic compounds, such as the higher molecular weight homologues, merely reflect their large affinity for the micelles and surfactant-coated stationary phase, compared to bulk aqueous component in the mobile phase. Solubility data indicate that these compounds are virtually insoluble in water. This finding implies that as the amount of such hydrophobic solutes in the aqueous phase is almost negligible, they can only be transported between the micelles in the mobile phase and the surfactant-modified stationary phase by a direct transfer process, and no partitioning will take place between aqueous phase and micelles, or between aqueous phase and stationary phase. Therefore, only a single equilibrium will exist, which is described by P_{SM} (Fig. 5.8) [29].

Although mentioned in theories of pseudo-phase chromatography, the possibility of a direct transfer of an insoluble or a sparingly water-soluble solute, from the micelle in the mobile phase to the surfactant-coated stationary phase, was largely ignored. For this situation, a modified form of the equation of Armstrong and Nome (eq. 5.1) was derived [29], which successfully accounts for the dependence between k and $[M]$, observed in the elution of such hydrophobic solutes:

$$k = \frac{\phi P_{SM}}{v[M]} \quad (5.18)$$

The drawback of the solubility limit theory is the difficulty in defining an exact limit for this behavior. Also, if the intercept in eq. 5.9 is very small, although not equal to zero, the binding constants K_{AM} and K_{AS} can be calculated, but the errors will be very high. Only P_{SM} values can be evaluated for very hydrophobic solutes. It was checked that this partition

coefficient, determined at any given micellar mobile phase composition from eq. 5.18, is useful to predict solute retention for other mobile phase concentrations with little error, over the entire alkylbenzene homologous series range [29].

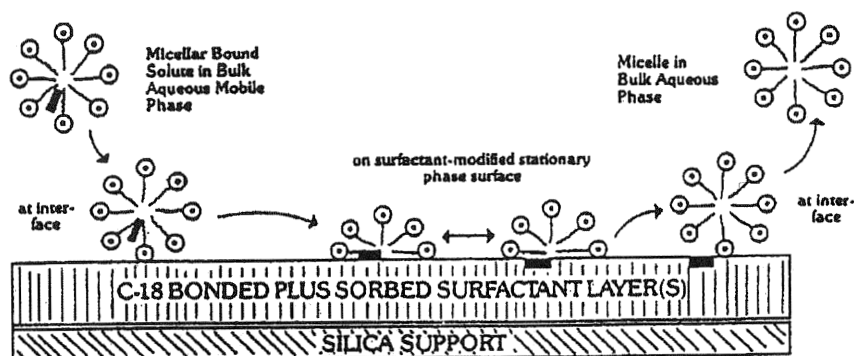


Figure 5.8 Representation of the direct transfer process for distribution of a solute between the micellar pseudo-phase and the surfactant-modified C18 stationary phase. Reprinted from Ref. 29 with permission of the American Chemical Society.

It can be observed, in Fig. 5.6, that at low alcohol concentration the retention mechanism is a direct transfer between the micelles and the modified stationary phase, and at the highest alcohol concentrations, perhaps owing to an enhancement in the solubility, the dissolved portion of solutes in bulk solvent interacts with both micelles and stationary phase.

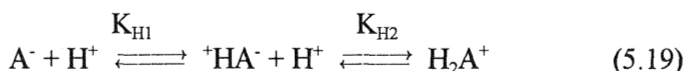
VI. EFFECT OF pH ON RETENTION

VI.1. *Changes in Acid-Base Behavior in Micellar Media*

The retention of weak organic acids and bases is affected by the pH of the micellar mobile phase. Solute-micelle binding constants of the dissociated and undissociated forms of a compound with anionic or cationic micelles are

different. Small changes in pH can thus significantly alter the chromatographic retention, particularly when the mobile phase pH is close to the value of the protonation constant ($\log K_H$). Therefore, for these compounds, the pH of the micellar mobile phase must be specified when retention data are reported. Knowledge of $\log K_H$ values in micellar media is crucial for a better understanding of the retention mechanisms and in designing optimization strategies in MLC.

The influence of micelles on the acid-base properties of analytes can be significant and is reflected in changes in $\log K_H$. A good example is the acid-base behavior of amino acids and peptides, which present the following equilibria [44, 45]:



Despite large structural differences among amino acids and peptides, their $\log K_H$ values in bulk aqueous solution are very similar. In micellar media, the effect of chemical structure on $\log K_H$ is much more pronounced (Table 5.4), which is indicative of the profound influence of solute-micelle interactions on the acid-base behavior of these compounds [44]. The apparent protonation constant in micellar media is expressed as:

$$K_{H,app} = K_H \frac{1 + K_{HAM}[M]}{1 + K_{AM}[M]} \quad (5.20)$$

where K_{AM} and K_{HAM} are the binding constants of the dissociated and undissociated species, respectively.

As shown in Table 5.4, both carboxylate and amine groups of amino acids and peptides are weaker acids in SDS micelles. The contributory factors to the positive $\log K_H$ shift in an SDS system are, first, the electrostatic repulsion between the carboxylate group and SDS micelles;

Table 5.4 Protonation Constants of Several Amino Acids and Peptides Measured Potentiometrically in Aqueous and Micellar Solutions [44].

Compound	Ionization Step	$\log K_{nm}^a$	0.1 M SDS		0.1 M CTAB	
			$\log K_m^b$	$\Delta \log K^c$	$\log K_m^b$	$\Delta \log K^c$
Glycine	1	9.74	9.72	-0.03		
	2	2.34	2.57	+0.23		
Aspartic acid	1	9.80	9.76	-0.04		
	2	3.45	3.68	+0.23		
	3	2.04	2.39	+0.35		
Glycyl-glycine	1	8.08	8.22	+0.14		
	2	2.89	3.34	+0.45		
Benzoic acid	1	4.15	4.76	+0.47	3.18	-0.97
Lysine	1	10.56	10.55	-0.01		
	2	9.32	9.78	+0.46		
	3	2.29	3.88	+1.59		
Phenylalanine	1	9.23	9.25	+0.02	8.71	-0.54
	2	2.21	4.07	+1.86	2.21	0.00
Lysyl-phenylalanine	1	10.45	10.26	-0.19		
	2	7.35	8.03	+0.68		
	3	2.76	4.94	+2.18		
Tryptophan	1	9.21	9.68	+0.57	8.18	-1.03
	2	2.32	4.59	+2.27	2.11	-0.21
Phenylalanyl-phenylalanine	1	- ^d	8.06	- ^d		
	2		4.73			

^a K_{nm} = Protonation constants in nonmicellar solution containing 0.1 M NaCl; ^b K_m = Protonation constants in micellar solution containing 0.1 M NaCl; ^c Shift of protonation constant; ^d The solubility was too low to be measured potentiometrically.

second, the hydrophobic and electrostatic attraction of the alkyl and protonated amine groups to the SDS micelles; and third, the localization of protonated weak acid, ^+HA , in an environment with lower dielectric constant. In addition, the molecular size (and/or aqueous solubility) of the compounds also seem to play a role in influencing binding constants, and consequently, the magnitude of the $\log K_H$ shift. As expected, the magnitude of the $\log K_H$ shift is larger for compounds experiencing greater interactions with SDS micelles (*i.e.*, $\log K_H = 1.6-2.3$ for amino acids and peptides with hydrophobic and basic groups, as compared with $\log K_H = 0.2-0.5$ for the more polar compounds).

In cationic CTAB solution, the shift sign of $\log K_H$ is opposite of that in SDS solutions [44]. The compounds are stronger acids in CTAB micellar media than in aqueous solutions. This is probably due to the strong electrostatic attraction of the negative charge on the conjugate bases, to the cationic head groups of CTAB micelles. In contrast to SDS, the shift in $\log K_{H1}$ is larger than in $\log K_{H2}$. The large $\log K_H$ shift of benzoic acid in CTAB solution as compared to the shifts observed for amino acids, and to the same compound in SDS solution, can be attributed to the strong electrostatic attraction and hydrophobic interactions of the conjugate base of benzoic acid, and perhaps more importantly, to the lack of electrostatic repulsion which is evident for the other solutes.

Apparent protonation constants were evaluated for several solutes in SDS micellar solutions, from MLC retention factors at varying concentration of micelles and 1-propanol [46]. It was observed that the modifier decreased the values of $\log K_{H,app}$, whereas these were increased with micelle concentration. The shifts in $\log K_{H,app}$ upon variation of modifier concentration are caused by both the modification of the thermodynamic constants in the aqueous-organic solvent, and the displacement of acid-base equilibria due to the modification of the interaction of the solute with the micelles, in the presence of modifier.

VI.2. *Consequences of Micellar Association on the Chromatographic Retention of Weak Acids and Bases*

A major constraint of silica bonded phases is the limited pH range of between 2.5 and 7.5. This is troublesome in the separation of poorly retained strong acids and bases, whose protonation constants are either outside or close to the boundaries of this range. In such cases, introduction of electrostatic interactions through the addition to the eluent of a small amount of surfactant, with a charge opposite to that of the solute, provides adequate retention. Thus, the use of micellar SDS mobile phases shifts the apparent protonation constants of solutes to milder pH conditions, and benefits the observation of the maximal limiting retention within the operable limits of silica-based columns. Also, the wider distribution of protonation constants of compounds in micellar media gives a greater selectivity to the chromatographic separations.

The pH of the micellar mobile phase is usually buffered using the phosphoric or citric acid-base systems. Potassium ion cannot be used with SDS, as potassium dodecyl sulfate precipitates from aqueous solutions due to its high Krafft point (see Chapter 2). The column should be equilibrated by purging with the mobile phase until the pH before and after the column is identical. Only one peak is observed in the chromatograms of weak acids and bases because prototropic equilibria are much faster than the solute-micelle or solute-stationary phase dynamics.

Cyano-bonded and C18 columns interact very differently with surfactant monomers, resulting in a different elution behavior of organic acids and bases, as a function of micelle concentration and pH in the mobile phase [47]. Cyano packings do not appear to adsorb SDS monomers and generally have the capability of retaining various ionic species. C18 columns are modified by adsorption of SDS monomers, with the negative head groups in contact with the mobile phase, such that the surface is charged and will repulse anionic species. Some examples of retention behavior for weak organic acids and bases are shown below.

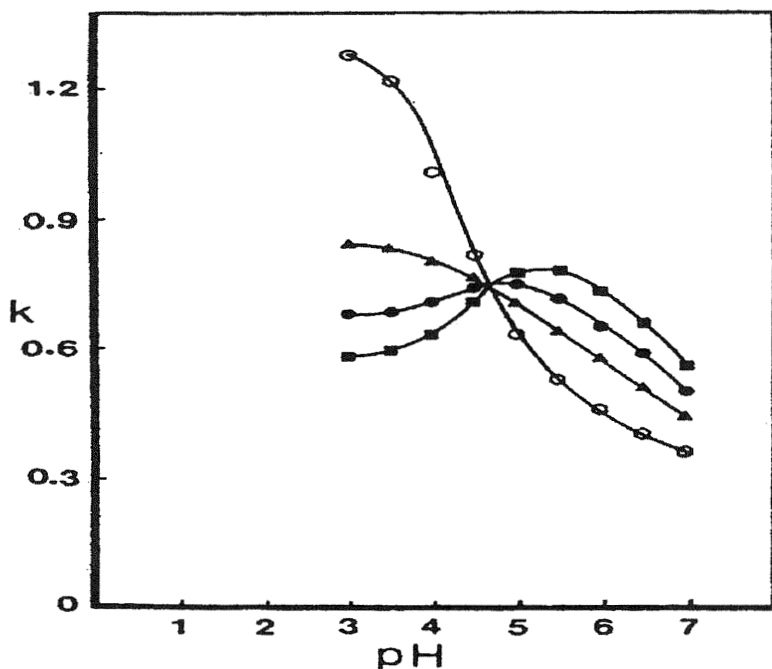


Figure 5.9 Change in chromatographic retention with pH for benzoic acid in a cyano-bonded column, at various SDS molar concentrations: (○) 0.02, (▲) 0.05, (●) 0.08, and (■) 0.10. Reprinted from Ref. 47 with permission of the American Chemical Society.

a) Cyano Columns

For some solutes, the pH of micellar solutions is of fundamental importance in determining the sign of the slopes in the $1/k$ vs. $[M]$ plots (positive, negative or zero). For benzoic acid at $\text{pH} < 4.5$, the retention factors decrease with increasing SDS concentration, because of hydrophobic interaction of the neutral acidic species with the SDS micelles, but at $\text{pH} > 4.5$, the retention factors increase owing to electrostatic repulsion between the negative charges of the basic species and micelles (Fig. 5.9) [47]. Hence, completely opposite behavior is observed depending on pH.

In the intermediate range of pH values, there is an isoelecting point where k is completely independent of SDS concentration. This is the pH at

which the two species, acid and base in equilibrium with each other, have the same retention. This is analogous to the isosbestic point in spectroscopy and would be expected to give the protonation constant in the micellar medium (for benzoic acid, $\log K_H$ is 4.2 in water, and 4.7 in SDS micellar solution, as obtained from the isoeluting point). It has been commented before, in Section II.2, that if solute retention is constant with increasing micelle concentration, this does not necessarily mean that the solute-micelle binding constant is zero. The isoeluting point (50% ionization point) clearly demonstrates that the lack of change in k , with micelle concentration in the mobile phase, is the result of the balancing or opposing hydrophobic and electrostatic effects caused by the change in the form of the species.

On the other hand, inspection of the retention factors for weak bases using cyano columns and SDS eluents shows that the largest k values occur in more basic solution, where the neutral free-base form is present, and the smallest in acidic solution where the protonated, positively charged form exists, which has favorable electrostatic attraction to the negatively charged micelles (Fig. 5.10) [47].

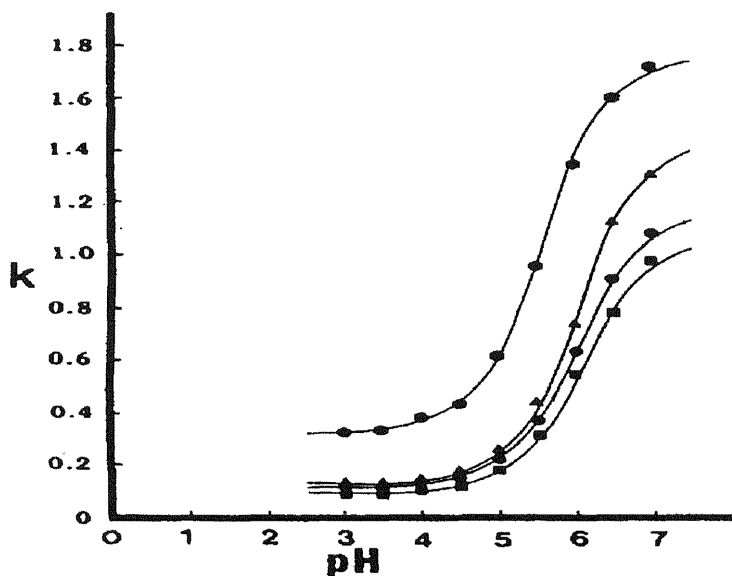


Figure 5.10 Change in chromatographic retention with pH for aniline in a cyano-bonded column, at various SDS molar concentrations: (◆) 0.02, (▲) 0.05, (●) 0.08, and (■) 0.10. Reprinted from Ref. 47 with permission of the American Chemical Society

b) C18 Columns

The effect of pH on the retention factors of solutes, eluted with an anionic surfactant, is very similar on C18 columns to that obtained using cyano columns, when hydrophobic interactions dominate. However, less hydrophobic and negatively charged solutes will elute very quickly on C18 columns, because of repulsion from both micelles and negatively charged modified stationary phase. Fig. 5.11 shows plots of k' vs. pH with the typical

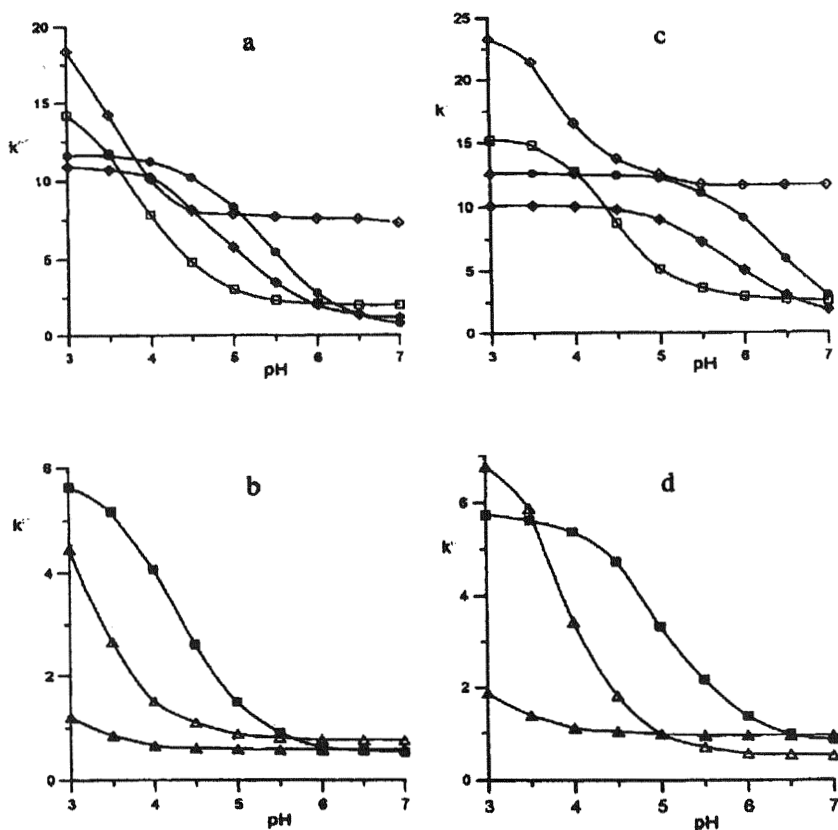


Figure 5.11 Modification of the retention with pH in a C18 column for: ($\diamond$) benzocaine, ($\square$) ethacrynic acid, ($\bullet$) xipamide, ($\blacklozenge$) bumetanide, ($\blacksquare$) furosemide, ($\triangle$) tyrosine, and ($\blacktriangle$) sulfanilamide, for 0.05 M SDS-8% (v/v) 1-propanol (a,b), and 0.15 M SDS without alcohol (c,d) mobile phases. Reprinted from Ref. 46 with permission of Elsevier.

behavior found when weak acids are eluted from C18 columns. Modification of the retention factors with pH is shown for mobile phases of 0.05 M SDS containing 8% (v/v) 1-propanol and without this modifier. Inspection of the curves reveals that the largest k values are achieved in acidic solutions, where the neutral or cationic forms of the solutes are present, and the smallest, in more basic solution where the anionic or neutral forms dominate. The dependence of k on pH at a constant concentration of surfactant and modifier is sigmoidal, which resembles that of conventional acid-base titration curves. The observed increase in retention at lower pH could be ascribed to the fact that the interaction of solutes with the surface of the surfactant-modified stationary phase is stronger than with micelles.

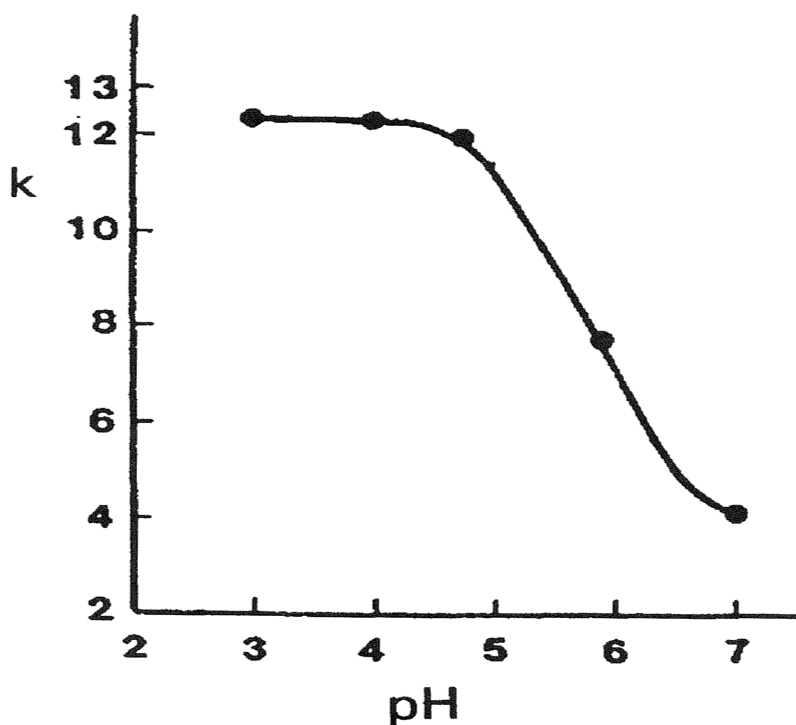


Figure 5.12 Change in chromatographic retention with pH for aniline in a C18 column with 0.05 M SDS mobile phases. Reprinted from Ref. 47 with permission of the American Chemical Society

Adsorption of anionic surfactant monomers on the surface of a C18 stationary phase also causes protonated organic bases to be retained a longer period of time than the neutral free-base forms, because of electrostatic attraction. Consequently, the elution behavior of protonated bases will mimic that of acids on C18 columns (Fig. 5.12), in contrast to the behavior observed on cyano columns. Figures 5.10 and 5.12 are mirror images of each other.

No isoeluting point exists with C18 stationary phases, but it can be assumed that $\log K_H$ equals the pH where the change in k per unit change in pH is maximum. It was found that the average $\log K_H$ values obtained from four different concentrations of SDS, with a C18 column, were in good agreement with those obtained from the isoeluting point using a cyano column [47].

VII. EFFECT OF IONIC STRENGTH ON RETENTION

Salts, such as NaCl, can also influence chromatographic retention. The Debye-Hückel equation predicts a decrease in activity coefficients with increasing ionic strength, with a concomitant increase in solute solubility (salting-in effect). In this context, the terms "salting-in" and "salting-out" apply to the solute becoming more or less soluble in bulk aqueous phase, respectively, and do not refer to the behavior in the micelle. Because the solubility of ionic organic species, as a function of salt concentration, depends on both their ionic and carbocyclic portion, it will be the result of a combination of electrostatic and hydrophobic effects. The behavior of antibinding and binding solutes should be considered separately.

VII.1. *Salt Effect in Antibinding Behavior*

If antibinding is mainly an electrostatic phenomenon, one would expect to see definite salt effects on this chromatographic behavior. In effect, modification of ionic strength might be sufficient to change completely an antibinding to a binding type behavior (*i.e.*, salting-out into the micelle). In the absence of

salt, bromophenol blue is an antibinding compound, whereas in the presence of as little as 0.02 M NaCl, it appears to be nonbinding. At slightly higher salt concentration, the compound binds strongly to SDS micelles [48]. In fact, binding of bromophenol blue to SDS micelles increases substantially more than might be expected from the linear increase in the concentration of NaCl.

The chromatographic behavior of bromophenol blue plotted as k vs. pH, with and without added salt, is shown in Fig. 5.13 [47]. Large differences in k occur at high pH, and the two curves tend to converge going towards low pH values, indicating that salt effects (electrostatic effects) should be larger for anionic species and smaller for neutral species. Also, the elution behavior vs. micelle concentration at high pH with salt reverses, compared to that without added salt. The bromophenol blue anion is less retained using 0.05 M SDS compared to 0.08 M SDS, but has the opposite behavior when NaCl is added, indicating that the anionic species in the presence of salt behave more like the neutral species (*i.e.*, the sign of the slope of the $1/k$ vs. $[M]$ plot changes from negative to positive).

For most antibinding solutes, the slope of $1/k$ vs. $[M]$ becomes less negative with increasing ionic strength, although not all compounds show this trend, indicating that more than simple electrostatic effects must be considered. For example, the interaction of naphthol green B with SDS micelles appears to be largely unaffected by the addition of salt. Conversely, the slope of $1/k$ vs. $[M]$ for thiocyanate ion becomes even more negative with increasing NaCl concentration [48]. Therefore, it seems that for the transition from antibinding to nonbinding and further for binding to occur, the solute ion must have sufficient hydrophobic character to associate with the nonpolar portion of the micelle, once electrostatic repulsions have been minimized.

In lyophobic colloidal systems, the addition of sufficient salt generally brings about flocculation. An analogy between colloidal flocculation and the nonbinding-binding transition has been drawn [48]. The counterion layer around the micelle is narrowed in a solution containing higher concentration of ions, which facilitates the approximation of the solute to the micellar assembly. Hydrophobic interactions between the solute and the nonpolar core of the micelle can then be established.

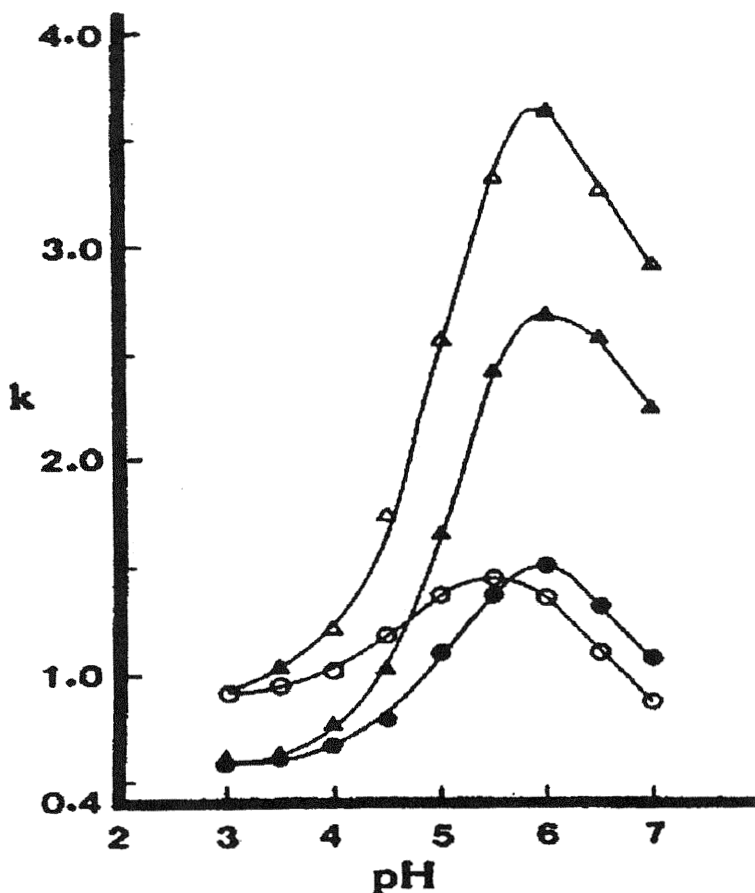


Figure 5.13 Salt effects on the retention factor of bromophenol blue in a cyano-bonded column, at various pH values. Micellar mobile phase: (○) 0.05 M SDS, (●) 0.08 M SDS, (△) 0.05 M SDS-0.10 M NaCl, and (▲) 0.08 M SDS-0.10 M NaCl. Reprinted from Ref. 47 with permission of the American Chemical Society.

The behavior of thiocyanate is difficult to explain in terms of electrostatic criteria. However, it may be possible to rationalize by considering the negligible hydrophobic character of this ion. When salt is added, the predominate factor may be the ions increased affinity for the bulk solution (salting-in effect), resulting in a lesser association with micelles and

decreased retention with increasing salt concentration. In contrast, many organic ions tend to be salted-out to the micelle.

VII.2. *Binding Solutes*

Only a few studies have been made on the effect of ionic strength on the behavior of binding solutes [36, 43, 49]. The main effect of NaCl is to decrease electrostatic interactions. The salting-out effect corresponds to a reduction of electrostatic repulsions by NaCl, but the addition of NaCl also decreases electrostatic attractions. In fact, no uniform behavior seems to exist with ionic solutes. The presence of salt has different effects on solute-SDS binding constants, depending on the nature of the compound. For benzene derivatives, in general, the binding constants increased in the presence of NaCl. In contrast, for naphthalene derivatives, these only increased for 1-naphthol, while for naphthalene and 2-naphthol, they decreased [36].

VIII. THERMODYNAMIC PROPERTIES BASED ON THE THREE-PHASE MODEL

The effect of temperature on the retention of compounds provides thermodynamic data that describe the chromatographic process. Dorsey et al. [13] proposed the use of high temperatures in MLC and applied the Van't Hoff equation to micellar mobile phases:

$$\ln k = -\frac{\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} + \ln \phi \quad (5.21)$$

where ΔH° and ΔS° are the standard enthalpy and entropy of transfer for the solute from the mobile phase to the stationary phase. This equation does not

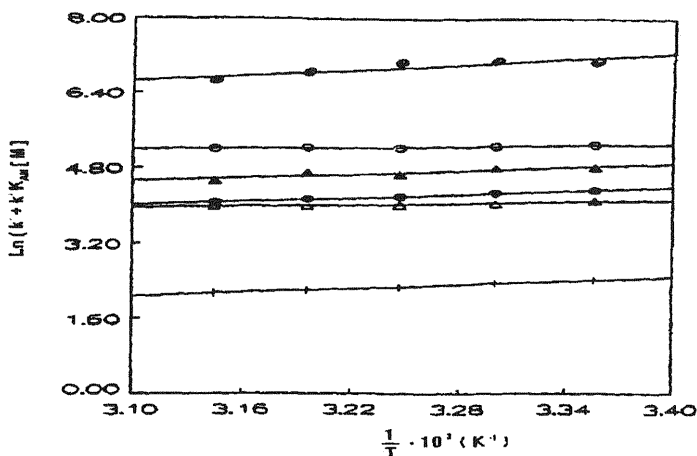


Figure 5.14 Van't Hoff plots for transfer of solutes from bulk water to the stationary phase for: (+) phenol, (Δ) benzene, ($\bullet$) anisole, ($\blacktriangle$) 1-naphthalenemethanol, ($\circ$) toluene, and ($\oplus$) naphthalene. Mobile phase: 0.10 M SDS. Reprinted from Ref. 50 with permission of the American Chemical Society.

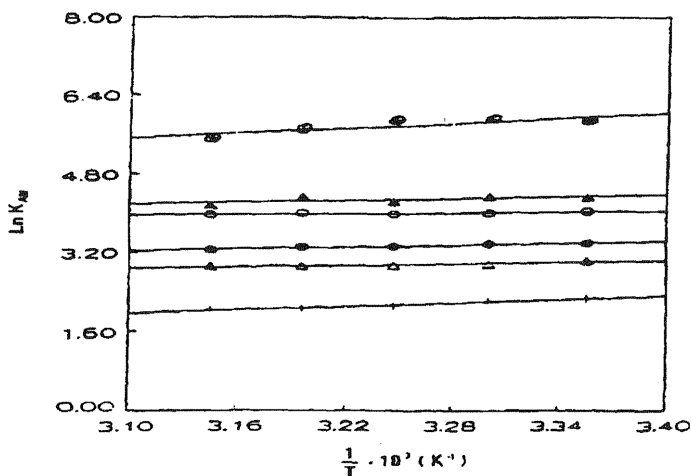


Figure 5.15 Van't Hoff plots for transfer of solutes from bulk water to micelles for: (+) phenol, (Δ) benzene, ($\bullet$) anisole, ($\circ$) toluene, ($\blacktriangle$) 1-naphthalenemethanol, and ($\oplus$) naphthalene. Mobile phase: 0.10 M SDS. Reprinted from Ref. 50 with permission of the American Chemical Society.

consider, however, the multiple equilibria involved in MLC, as described by the three-phase model. Tomasella et al. [34,50] proposed a more rigorous approach that accounts for the two main equilibria, and provides the thermodynamics associated with each transfer.

The transfer of solutes from bulk aqueous phase to the stationary phase is described by:

$$\ln [k (1 + K_{AM} [M])] = -\frac{\Delta H_{AS}^{\circ}}{RT} + \frac{\Delta S_{AS}^{\circ}}{R} \quad (5.22)$$

If the standard enthalpy, ΔH_{AS}° , and the standard entropy, ΔS_{AS}° , are independent of temperature over the temperature range of interest, a plot of $\ln [k (1 + K_{AM} [M])]$ vs. $1/T$ will be linear (Fig. 5.14). ΔH_{AS}° does not change with increasing concentration of surfactant, since micelles have no effect on this transfer. The general trend is a more negative ΔH_{AS}° with increasing hydrophobicity of the compounds, as observed for a group of PAHs (Table 5.5) [51]. This means that the transfer from bulk aqueous phase to stationary phase becomes enthalpically favored for more hydrophobic compounds. The less negative values of ΔH_{AS}° found in MLC, with respect to conventional RPLC, indicate that the effect of temperature on k is less pronounced with micellar eluents.

In addition, the change in enthalpy for the transfer of solutes from bulk aqueous phase to the micellar pseudo-phase can be calculated by the following equation:

$$\ln K_{AM} = -\frac{\Delta H_{AM}^{\circ}}{RT} + \frac{\Delta S_{AM}^{\circ}}{R} \quad (5.23)$$

The plot of $\ln K_{AM}$ vs. $1/T$ gives a straight-line whose slope and intercept will provide ΔH_{AM}° and ΔS_{AM}° (Fig. 5.15). Again, ΔH_{AM}° becomes usually greater with increasing compound hydrophobicity. However, a change in

temperature displays a minor change in K_{AM} , when compared with the changes that are caused by the addition of a few percent of organic modifier to the mobile phase.

Table 5.5 Change in Enthalpy (Kcal/mol) for Transfer of Solutes from Bulk Aqueous Phase to the Stationary Phase and from Bulk Aqueous Phase to Micelles [51].

Compound	ΔH_{AS}°	ΔH_{AM}°
Naphthalene	-4.49	-2.65
Acenaphthylene	-6.20	-4.43
Fluorene	-4.84	-3.08
Anthracene	-8.19	-5.40
Phenanthrene	-8.76	-6.58
9-Methylanthracene	-9.05	-7.05
Fluoranthene	-8.58	-6.12
Pyrene	-9.29	-6.88
Chrysene	-13.3	-10.5
Benzo[a]anthracene	-12.2	-9.68
Benzo[b]fluoranthene	-13.9	-11.4
Benzo[a]pyrene	-14.9	-11.8
Benzo[e]pyrene	-12.8	-10.6
Perylene	-14.1	-11.7

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6

The Efficiency Problem

I. INTRODUCTION

The first historical use of micellar phases in chromatography was with the Gel Permeation Chromatography technique (GPC) (see Chapter 3). The low efficiency problem was not noted in the early use of micellar phases because GPC is not a technique that produces sharp peaks. However, Armstrong observed very early that the TLC micellar spots were broader than nonmicellar ones [1]. Broad peaks, *i.e.*, poor efficiency, were obtained when micellar mobile phases were used instead of classical hydro-organic mobile phases in classical LC [2, 3]. Dorsey attributed the reduced micellar efficiency to a poor wetting of the apolar stationary phase by the aqueous surfactant phase [3]. He proposed the addition of 3% 1-propanol to remediate the problem [3, 4]. Yarmchuck et al. thought that a slow mass transfer between the micelles and the stationary phase was responsible for reduced efficiency [5]. Armstrong [6], Berthod [7] and Hinze [8] concluded that poor mass transfer within the stationary phase itself was mainly responsible for the observed low efficiency. Two main approaches were proposed to enhance the efficiency in MLC: (i)-the addition of low amounts (3% v/v) of 1-propanol [3] to decrease the amount of adsorbed surfactant and to increase the stationary phase wetting, (ii)-to raise the temperature [4, 5] to reduce the liquid viscosity and to increase the chemical reaction rates.

In this chapter, the possible causes of the reduced efficiency in MLC will be surveyed and a remediation path will be proposed and discussed. The chromatographic process is rapidly exposed pointing out the thermodynamics (retention time) and the kinetics (peak efficiency). The differences between a classical hydro-organic reversed mobile phase and a micellar phase are recalled. The kinetics of the chromatographic process can be modeled using the Knox equation that relates the reduced plate height to the

reduced mobile phase velocity. Knox plots with classical hydro-organic mobile phases and micellar mobile phases are compared and discussed. Surfactant adsorption on the stationary phase and solute exchange between micelles and the bulk are the two main differences between classical and micellar phases. These two mechanisms are likely responsible for the major part of the efficiency loss. The effect of the addition of various organic solvents on the micelle physicochemical structure and surfactant adsorption is exposed. The efficiency enhancements obtained with temperature changes and/or organic modifier additions are described.

II. CHROMATOGRAPHIC PROCESS AND EFFICIENCY

II.1. Thermodynamics

The thermodynamics of the chromatographic process is responsible for the solute retention. The retention volume, V_R , is related to the solute affinity constant for the stationary phase, K , by

$$V_R = V_O + KV_S \quad (6.1)$$

in which V_O and V_S refer to the mobile and stationary phase volume inside the column. The retention factor, k , is expressed by

$$k = (V_R - V_O)/V_O = K\phi \quad (6.2)$$

where $\phi (=V_S/V_O)$, is the phase volume ratio.

The peak retention volume and factor are due to chemical equilibria which are temperature dependent. The classical Gibbs free energy equation can be applied:

$$\Delta G^\circ = -RT \ln K = \Delta H^\circ - T\Delta S^\circ \quad (6.3)$$

Combining eqs. 6.2 and 6.3 yields:

$$\text{Ln } k = -\Delta H^{\circ}/RT + \Delta S^{\circ}/R + \text{Ln } \phi \quad (6.4)$$

The plots of $\text{Ln } k$ versus $1/T$, the Van't Hoff plots, will be linear if ΔH° and ΔS° are constant over the temperature range studied. The slope of the Van't Hoff plot gives the enthalpy change associated with the solute transfer from the mobile to the stationary phase. The intercept is related to the entropy change. Nonlinearity of these plots would indicate changes in the chromatographic mechanism with temperature.

II.2. Kinetics

The kinetics of the chromatographic process is linked to efficiency. The number of theoretical plates, N , that measures column efficiencies, is best estimated using the Foley-Dorsey equation when a departure from the Gaussian symmetrical peak shape is noted [9]:

$$N = \frac{41.7(t_R/W_{0.1H})^2}{B/A + 1.25} \quad (6.5)$$

in which $W_{0.1H}$ is the peak width at 10% peak height, B/A is the peak asymmetry factor measured by the ratio of the back (B) to front (A) half portion of the $W_{0.1H}$ peak width referring to its t_R retention time. This equation takes into account the peak asymmetry. In MLC, the efficiency decreases are always associated with peak tailings, i.e., asymmetry. All plate counts given were obtained using the Foley-Dorsey equation.

According to Snyder and Kirkland [10], the contributions to band broadening in a column can be represented in H , the column plate height, by the equation:

$$H = C_e d_p + \frac{C_m d_p^2 u}{D_m} + \frac{C_d D_m}{u} + \frac{C_{sm} d_p^2 u}{D_m} + \frac{C_s \theta_f^2}{D_s} \quad (6.6)$$

in which the C values are constant plate height coefficients related to eddy diffusion (e), mobile phase mass transfer (m), longitudinal diffusion (d), stagnant mobile phase mass transfer (sm) and stationary phase mass transfer (s), θ_f is the thickness of the stationary phase layer, D_m the solute diffusion coefficient in the mobile phase, D_s the solute diffusion coefficient in the stationary phase layer and u is the mobile phase velocity.

The Knox equation [11] is used in LC to determine the contributions to the final solute band width. It can be expressed as:

$$h = A' v^{1/3} + B'/v + C'v \quad (6.7)$$

where A' , B' and C' are the constants of the Knox equation, h is the reduced plate height calculated as $h=H/d_p$, where H is the column plate height ($H=L/N$, L being the column length and N the number of theoretical plates), d_p is the stationary phase particle diameter, v is the reduced mobile phase velocity, i.e., $v = ud_p/D_m$, with the average mobile phase velocity, u , in cm/s and D_m in cm²/s.

The A' , B' and C' terms are related to flow anisotropy, molecular longitudinal diffusion and mass transfer processes, respectively. The theoretical support for the Knox equation was derived by Horvath [12]. The A' term cannot be expressed simply. The theoretical treatment links A' to structural parameters of the column packing, porosity, pore volume, pore diameter and tortuosity [12]. A' is related to the flow pattern and the general band spreading due to "eddy" diffusion [13]. The B' term (longitudinal molecular diffusion) was written as [13]:

$$B' = 2[\gamma_m + \gamma_s (D_s/D_m)k] \quad (6.8)$$

where γ_m and γ_s are obstruction factors for diffusion through granular and/or porous material. The C' term of the Knox equation represents the mass transfer contribution to solute band broadening. It was written as [13]

$$C' = q \left(\frac{k + \psi}{1 + k} \right)^2 \left(\frac{D_m}{\gamma_{sm} \psi D_m + k \gamma_s D_s} \right) \quad (6.9)$$

where the γ terms are obstruction factors with the subscripts defined for eq. 6.6, ψ is the stagnant mobile phase fraction and q is a geometrical factor depending on porosity [12-14].

Band broadening and/or unusual retention are also known to occur when specific interactions take place between particular solutes and the stationary phase. The most common cause of such band broadening is a stationary phase overload. It can be also an exclusion phenomenon due to the small size of the stationary phase pores and the large size of the solute. Acid-base interactions between the surface silanols and basic compounds, especially the amine compounds, dramatically broaden the corresponding peaks. Peak deformations, either frontings or tailings, are observed.

II.3. External Band Broadening

The plate number, i.e., the chromatographic efficiency, is a parameter difficult to estimate correctly [15]. The use of methods assuming Gaussian peaks may produce grossly overestimated peak efficiency. The asymmetry-based method [9] (eq. 6.5) was designated as the manual method giving plate numbers closest to the exact plate numbers obtained with the moment method [15, 16]. The second central reduced moment of a peak corresponds to its variance [17]. It is known, but too often overlooked, that the overall peak variance, σ^2 , is a combination of the column variance plus all the other extra-column variances

$$\sigma^2 = \sigma_{\text{col}}^2 + \sigma_{\text{ct}}^2 + \sigma_{\text{inj}}^2 + \sigma_{\text{det}}^2 + \sigma_{\text{other}}^2 \quad (6.10)$$

where the subscripts refer to the column, connecting tubing, injector, detector and other variance contributions (e.g., electronic time constants, frits, unions).

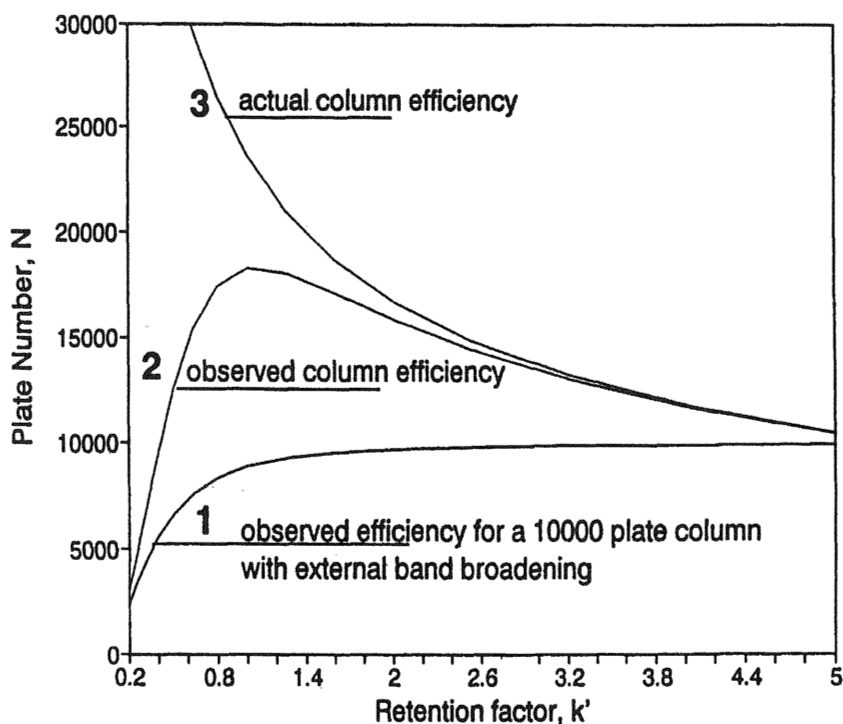


Figure 6.1 Efficiency experimental measurement. 1- observed efficiency for a 10,000 plate column, the extra-column band broadening is heavy in decreasing the column efficiency for low k solutes. 2- observed efficiency with extra-column band broadening and efficiency enhancement combined with k reduction, a maximum N value seems to exist. 3- true column efficiency when enhancements are used in MLC. Reprinted from [25] with permission of Elsevier.

It is possible and desirable to reduce all extra-column variances. However, external band broadening cannot be fully eliminated. The measured variance is always the sum of the actual column variance, σ_{col}^2 , and a

constant term. If the column efficiency is constant for a variety of solutes, the square root of the column variance, σ_{col} , increases linearly with time or elution volume. The other variance contributions are rather constant with time. As the retention time increases, the external band broadening contribution to the global variance becomes less significant. The measured efficiencies of highly retained peaks appear higher than the efficiencies of low k peaks. Figure 6.1 (curve 1) shows that a plateau of constant efficiency, the true column efficiency, is rapidly reached. Most methods for improving the efficiency in MLC reduce the retention factor at the same time that they decrease the σ_{col}^2 parameter. Then, the experimental observed efficiency may show a maximum value [12] for a given k value as illustrated by Figure 6.1 (curve 2). It means that the efficiency enhancement can be annihilated by the extra-column effects. This is specially true when nonoptimized chromatographic systems are used.

III. CHROMATOGRAPHIC PROCESS AND MICELLAR PHASES

The three phase model was exposed in the previous chapter. Basically, besides the solute exchange between the stationary and the mobile phase, the micelles introduce a secondary chemical equilibrium. Each equilibrium, micelle-aqueous phase, stationary phase-aqueous phase and stationary phase-micelles, has its thermodynamic constant, P_{WM} , P_{WS} and P_{SM} , respectively, linked together and to the micellar concentration, $[M]$, the phase ratio, ϕ , and the surfactant molar volume, v , by the Armstrong equation [18].

$$\frac{1}{k} = \frac{1}{P_{WS}\phi} [1 + v(P_{WM} - 1) [M]] \quad (6.11)$$

The P_{SM} constant, not contained in eq. 6.11, is the ratio of the two other constants [18]:

$$P_{SM} = P_{WS}/P_{WM} \quad (6.12)$$

Also, each equilibrium is not reached instantaneously and has its own kinetic constant. All three kinetics constants intervene in the global observed efficiency and are studied individually thereafter.

III.1. *Micelle-Aqueous Phase Exchanges*

In the early studies, the micellar reduced efficiency was attributed to a diminished rate constant for solute exit from the micellar aggregates [5]. If the solute stays inside the micelles that move with the mobile phase for a long time, it interacts with less stationary phase. It "sees" a shorter column. Assuming the solute entrance in the micelle is diffusion controlled and equivalent for all solutes, the exit rate constant is inversely proportional to the P_{WM} constant. The solutes with the highest affinity for the micellar phase should be the least retained. Actually, they are the most retained solutes which shows that the solute affinity for the micelles (P_{WM}) are strongly correlated to the affinity for the surfactant covered stationary phase (P_{WS}). These combined effects with the hydrophobic solutes show that they are the ones with the poorer efficiency [5]. The reduced solute exit rate from the micelle produces a decreased solute diffusion coefficient in the mobile phase, D_m .

It was shown that the D_m diffusion coefficient was decreased by micelle inclusion related to the P_{WM} constant [6]. The overall solute diffusion coefficient, D_m , in a micellar solution depends on the micelle diffusion coefficient, D_M , and the solute diffusion coefficient in the aqueous phase, D_W ,

$$D_m = \frac{D_M}{1 + \Psi} + \frac{D_W}{1 + \frac{1}{\Psi}} \quad (6.13)$$

in which Ψ is related to the solute affinity constant, P_{WM} , measured by MLC:

$$\Psi = \frac{1 - [M]v}{P_{WM} [M]v} \quad (6.14)$$

The product $[M]v$ is the volume percent of micellar phase and $1-[M]v$ is the volume percent of the aqueous phase [6].

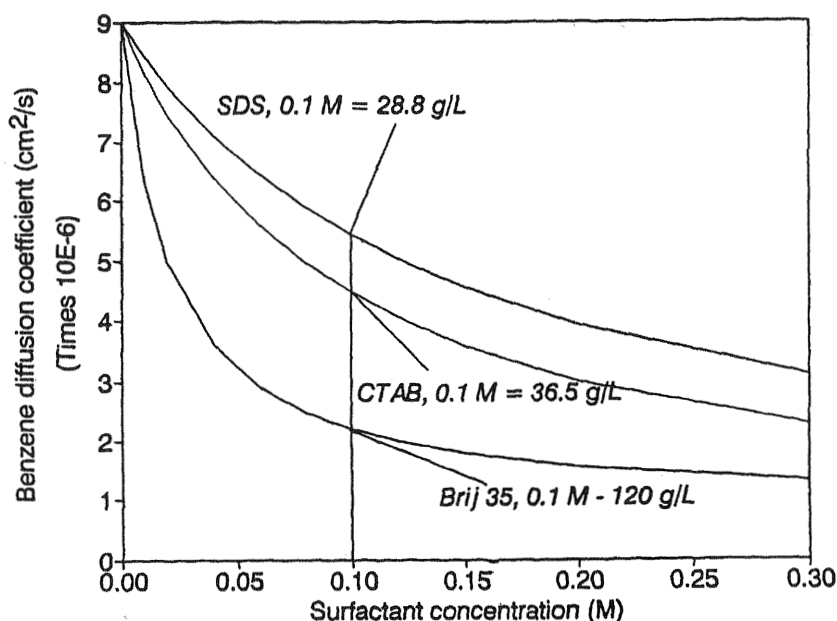


Figure 6.2 Decreases of the benzene diffusion coefficient due to its inclusion in micelles of different surfactants (Data from [6]). Reprinted from [25] with permission of Elsevier.

Figure 6.2 illustrates the decrease of the benzene diffusion coefficient in various micellar solutions. Table 6.1 lists the diffusion coefficients of various solutes in different micellar media. Depending on the solute affinity for the micellar phase, the D_m diffusion coefficient can be decreased by a factor varying from 2 to 10 (Figure 6.2). Such a D_m decrease may highly increase the B' and C' terms of the Knox equation as

shown by eqs. 6.8 and 6.9, respectively. The corresponding reduced velocity, u , is also increased by the D_m decrease which should reduce the observed negative effect at low flow rates and magnify it at high flow rates. An important surfactant concentration does not seem to be favorable for efficiency.

Table 6.1 Diffusion Coefficients in Various Micellar Phases at 24°C

Solute	P_{WM}	P	D_m ($\times 10^{-6}$ cm ² /s)
<i>Brij 22, 5% w/v; 0.08 M</i>			
Benzyl alcohol	16.5	1600	5.1
Benzaldehyde	21.6	2100	4.7
Benzene	72.2	7000	2.9
Micelle	-	-	0.87
<i>Brij 35, 5% w/v; 0.042 M</i>			
Benzyl alcohol	10	400	6.3
Benzaldehyde	16	640	5.5
Benzene	45	1800	4.4
Micelle	-	-	0.94
<i>SDS, 1.4% w/v; 0.05 M</i>			
Benzene	77.4	4800	6.8
Toluene	242	15,000	3.4
Ethylbenzene	694	43,000	1.7
Propylbenzene	1940	120,000	0.94
Micelle	-	-	0.57

P is the partition coefficient referring to the micellar phase ($=P_{WM} \times N_{aggr.}$).

Brij 22 = C12E10, mw = 610, ν = 0.9542 mL/g or 0.582 L/mol, $N_{aggr.}$ = 97, cmc = 0.09 mM. **Brij 35** = C12E23, mw = 1200, ν = 1.064 L/mol or 0.867 mL/g, $N_{aggr.}$ = 40, cmc = 0.1 mM. **SDS**, mw = 288, ν = 0.246 L/mol or 0.854 mL/g, $N_{aggr.}$ = 62, cmc = 8.2 mM. Data from [7].

III.2. Micellar Phase- Stationary Phase Exchanges

Chapter 4 extensively described the surfactant adsorption occurring when micellar phases are used. This adsorption takes place with any surfactant,

ionic (see Figures 4.2 and 4.4) as well as nonionic (Figure 4.1). The surfactant adsorbed layer changes the solute-stationary phase and micelle-stationary phase interaction and kinetics. The amount of adsorbed material depends on the surfactant and the stationary phase used. It is about $4.5 \mu\text{mol}/\text{m}^2$ ($\sim 1.3 \text{ mg}/\text{m}^2$ or $\sim 140 \text{ mg}/(\text{g of C18 phase})$ or 14%) for the SDS surfactant on a C18 phase. Similar measurements gave $4.3 \mu\text{mol}/\text{m}^2$ ($\sim 2 \text{ mg}/\text{m}^2$ or $\sim 210 \text{ mg}/(\text{g of C18 phase})$ or 21%) for the CTAB surfactant on the same C18 phase (see Chapter 4). These amounts of adsorbed SDS or CTAB are close to two surfactant molecules per bonded moiety for the C18 phases. Corresponding results were obtained for nonionic surfactants [8, 19]. The adsorbed surfactant molecules fill up part of the silica pore volume and in doing so, reduce the stationary phase surface area [19, 20]. The surfactant adsorbed layer increases the thickness of the stationary phase organic layer, σ_f . Assuming a regular layer, the previously indicated adsorbed mass of SDS on the C18 phase corresponds to $1.06 \text{ mm}^3/\text{m}^2$ or an average thickness of about 1.06 nm. The same calculation for the CTAB layer on the C18 phase gives a thickness of about 1.6 nm. These estimated breadths roughly correspond to $\sim 1/2$ of the surfactant molecule lengths. The σ_f parameter intervenes quadratically in the last term of eq. 6.6 that means the surfactant layer may dramatically reduce the chromatographic efficiency.

III.3. Use of the Knox Plot to Investigate the Causes of Reduced Efficiency

As shown by eqs. 6.7, 6.8 and 6.9, the A' , B' and C' terms of the Knox equation are not directly related to the kinetic constants of the three equilibria occurring with micellar mobile phases. The slow exchange of the solute between the micellar and aqueous phases increases the three terms of the Knox equation. It increases the flow anisotropy (A' term). It reduces the molecular diffusion increasing the B' term (eq. 6.8) and the C' term (eq. 6.9). The reduction of the stationary phase-micellar phase solute-exchanges due to the adsorbed layer acts mainly on the C' term.

Table 6.2 lists the A' , B' and C' values experimentally obtained by several workers [19-22]. To be meaningful, the Knox plots should be obtained on the same column, with the same solute and different mobile

phases. In column #1, with the benzene solute, the efficiency loss is due to A' increases (+300%) and B' increases (+700%) compared to the values

Table 6.2 Parameters of the Knox Equation

Solute	Mobile Phase	Column	Optimal	A'	B'	C'	k
			Flow mL/min				
<i>Benzene</i>							
25°C	MeOH-water 70/30 v/v	1	0.2	1.1	3.1	0.2	1.1
	SDS 0.05M (1.4% w/v)	1	0.3	3.2	20	0.2	15.6
	ACN-water 30/70	2	1.2	0.65	16.4	0.026	17.3
	Brij 35 0.042M (5% w/v)	2	<0.1	1.0	6	0.03	26.1
	SDS 0.05M (1.4% w/v)	3	0.2	3.4	5.6	0.16	17.8
	45°C	SDS 0.05M (1.4% w/v)	3	0.7	2.7	11.4	0.05
<i>Ethylbenzene</i>							
	MeOH-water 70/30 v/v	1	0.2	1.1	3.8	0.15	3.5
	SDS 0.05M (1.4% w/v)	1	0.3	3.1	23	0.5	45
<i>Coumarin</i>							
	SDS 0.01M (0.3% w/v)	2	0.1	5.4	5.8	0.26	-
	SDS 0.01M+2% C3OH	2	0.2	4.0	8.5	0.018	-
	SDS 0.01M+1% C5OH	2	0.3	3.0	8.8	0.007	-

Column #1: 10 cm, 4.6 mm i.d., Radial-Pack ODS, 10 μ m (Waters), data from [21].

Column #2: 10 cm, 4.6 mm i.d., Apex ODS, 5 μ m (Jones), data from [21].

Column #3: 10 cm, 4.6 mm i.d., C14 bonded phase, 5 μ m, 3.5 μ mol/m², data from [22].

Room temperature, unless otherwise indicated.

obtained with the methanol-water mobile phase. The C' term is little changed. With the ethylbenzene solute, the A' and B' values are similar to the benzene values but the C' term is also increased by 350%. In column #2, the efficiency reduction compared to the acetonitrile (ACN)-water phase is due to moderate increases of both the A' (+54%) and C' (+16%) terms; the decrease of the B' term is not significant considering the raw data set [21]. In column #3, the hydro-organic reference values were not given. Assuming the column #1 values can be used, the efficiency loss is again due to the A' and B' term increases (+300% and +80%, respectively). When the temperature was raised from 25°C to 45°C, this decreased the A' and C' terms by -21% and -70%, respectively. The effect of alcohols on

the Knox parameters is seen by a decrease of the A' terms by -26% and -45% and a dramatic decrease of the C' parameters by -93% and -97% is shown when only 2% v/v propanol and 1% v/v pentanol, respectively, were added [22].

The use of the Knox plots to study the causes of micellar reduced efficiencies leads to the following conclusions. The micellar phase flow anisotropy seems to be much higher than the flow anisotropy obtained with a hydro-organic phase of comparable viscosity (increased A' term). This is only partly due to the micellar viscosity. The main reason of such differences in flow patterns is the partial clogging of the stationary phase pores by adsorbed surfactant molecules [19, 22]. A temperature raise decreases the mobile phase viscosity and the amount of adsorbed surfactant [22]. Both effects decrease the flow anisotropy and the A' term. It will be exposed thereafter that alcohol additions to a micellar phase dramatically reduce the amount of adsorbed surfactant.

The B' term is linked to solute diffusion coefficient which is reduced by micelle inclusion (eq. 6.8 and Figure 6.2). The increase of the B' term in micellar phases is due to the D_m change and also to the k increase (Table 6.2). Considering the C' term equation (eq. 6.9), it should be expected that micellar phases decrease C' because k values are higher and D_m coefficients are lower. This is not observed experimentally. It means that the surfactant adsorption causes dramatic decreases of the D_s parameter. The solute diffusion coefficient in the surfactant covered stationary phase is very difficult [6, 7, 20-21]. It slows down the solute mass-transfer. This effect becomes dominant for lipophilic solutes that have a high affinity for the stationary phase (ethylbenzene, Table 6.2). The peaks corresponding to lipophilic solutes become very broad with micellar mobile phases. Both a temperature raise and alcohol addition decrease the amount of adsorbed surfactant [19, 23]. Both actions reduce the C' term and improve the observed efficiency.

Clearly, the Knox plot study points out that surfactant adsorption on the stationary phase is responsible for the bulk of efficiency loss observed using micellar phases. A slow solute exchange between the micelle apolar core and the aqueous phase is another possible explanation for MLC efficiency loss.

IV. REMEDIATION OF REDUCED EFFICIENCY

The serious waste of efficiency in MLC was immediately noticed. It was a major drawback hindering the development of the technique. To be useful for the practicing chromatographer, the efficiency observed in MLC must at least approach that of conventional HPLC. As early as 1983, Dorsey et al. suspected a slow mass transfer coming from a poor wetting of the stationary phase by the aqueous micellar phase. They proposed the addition of 3% v/v 1-propanol to the micellar phase and heating the chromatographic system to 40°C [3]. The dramatic enhancement of the MLC efficiency triggered numerous studies of the effect of organic additives and temperature in MLC.

IV.1. Experimental Results

a) Polar Solutes with an Amino Group

β -Blockers were recently separated by MLC on a classical C18 phase with an SDS micellar mobile phase [24]. These experimental results are typical and can be used to illustrate the efficiency problem in MLC. Most β -blockers are amino-alcohol derivatives. They are difficult to quantify by classical LC because their amino group is positively charged. Interactions with the negative residual silanols of the stationary phase produce some peak tailing. The problem is easily solved by the addition of a small amount of triethylamine to the hydro-organic mobile phase that quenches the silanols. The use of dedicated columns for basic compounds with very few residual silanol groups is another elegant solution. MLC can also be used to separate and quantify these compounds. Table 6.3 lists the experimental chromatographic parameters obtained for 4 β -blockers [24]. Different alcohols were checked as possible efficiency improvers. Figure 6.3 illustrates these results.

As described extensively in Chapters 7 and 8, any alcohol addition to a micellar phase increases the elution strength. The full lines of Fig. 6.3 correspond to the peak efficiency; the dotted lines show the corresponding retention factors. A significant efficiency improvement (from 5 to 15 times increase) is obtained with the addition of 15% v/v propanol to the SDS

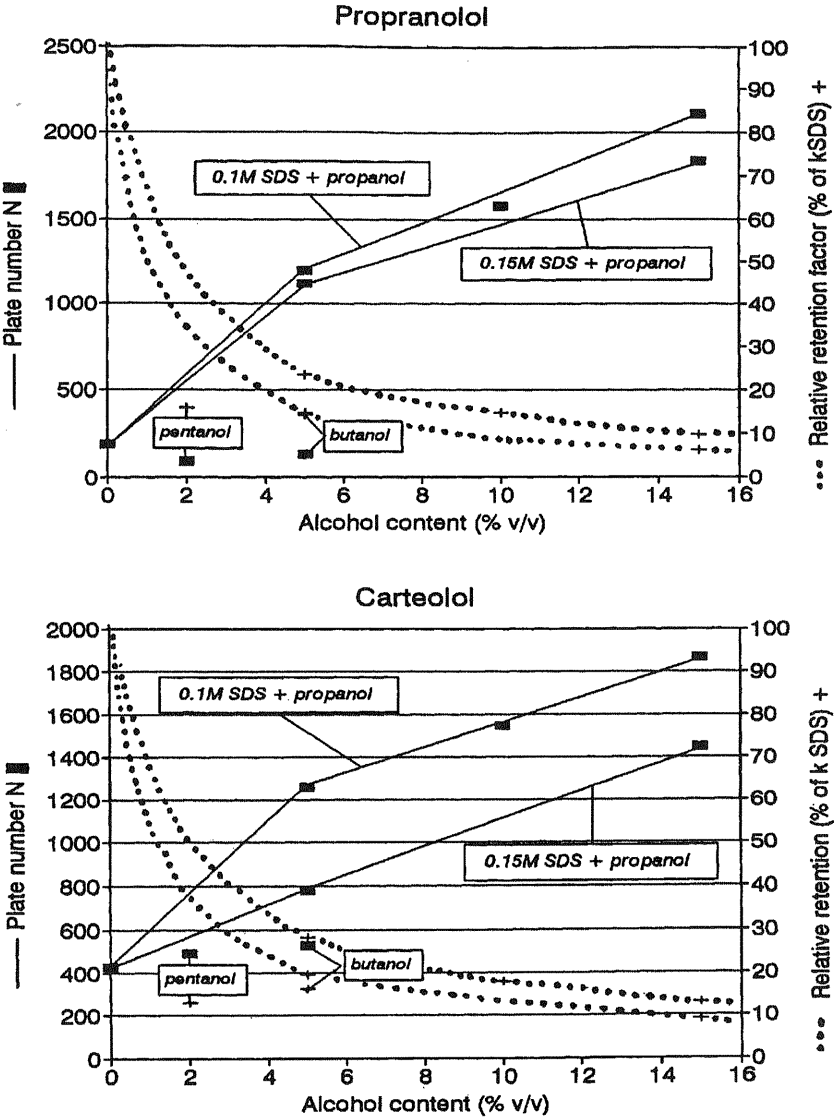


Figure 6.3 Combined changes produced by alcohol addition on efficiency (full lines) and retention factor (dotted lines) of two β -blockers: Propranolol (top) and Carteolol (bottom) (Data from [24]).

Table 6.3 Retention Factor and Associated Efficiency of the β -blockers' Peaks Eluted with Alcohol Containing Micellar Phases

Compound	Acebutolol		Carteolol		Celiprolol		Propranolol	
<i>Mobile Phase</i>	k	N	k	N	k	N	k	N
SDS 0.1 M	47	100	39	430	96	70	196	190
+propanol 5%	16	940	11	1260	24	510	46	1200
+propanol 10%	10	1200	7	1550	14	1050	29	1500
+propanol 15%	8	1550	5	1900	10.5	1150	19	2100
+butanol 5%	10	300	6.5	550	13	300	28	130
+pentanol 2%	7.5	300	5	500	9.5	240	31	90
SDS 0.15 M								
+propanol 5%	10.5	700	7.5	800	16	400	29	1100
+propanol 15%	5.5	1400	3.5	1450	7	1100	12	1850

Data from [24].

micellar phase. Butanol and pentanol were also used as organic additives (Table 6.3). The addition of 5% v/v butanol to the 0.1M SDS phase can enhance the peak efficiency up to four times (celiprolol), it can decrease it as well (propranolol). A 2% v/v pentanol addition has a similar effect on both retention and efficiency than the addition of 5% v/v butanol (Figure 6.3 and Table 6.3). The huge decrease in the β -blocker retention produced by the 2% v/v pentanol (and 5% v/v butanol) addition is due to a P_{WS} decrease [24]. The alcohol seems to reduce significantly the amount of adsorbed SDS on the stationary phase. When the negative SDS adsorbed ions decrease, the positive β -blockers are less retained.

b) Apolar Solutes

Benzene, toluene, 2-ethylanthraquinone (EAQ) and acetophenone were used as test solutes of low or medium polarity [8, 20, 23]. The linear alcohols from methanol to hexanol were studied as organic additives with SDS micellar phases [25]. Table 6.4 lists the P_{WM} and P_{WS} values (actually the ϕP_{WS} is listed since the phase ratio was not given) obtained for three of these solutes with different micellar phases. Clearly, the alcohol additions produce a parallel variation of the two constants P. It means that these additions

change the solute interactions with the micelles and the stationary phase in a comparable manner [24, 26].

Table 6.4 Effect of Alcohols on the Solute Affinity for the Micellar and Stationary Phases

Solute	Medium	P_{WM}	ϕP_{WS}
Benzene	SDS	90	60
	SDS + 4% propanol	68	50
	SDS + 4% butanol	61	32
	SDS + 4% pentanol	48	23
	SDS + 3% hexanol	24	15
	Brij 35	50	85
	Brij 35 + 15% ethanol	39	40
Acetophenone	SDS	55	49
	SDS + 4% propanol	56	23
	SDS + 4% butanol	31	11
	SDS + 4% pentanol	22	8
	SDS + 3% hexanol	16	6
	Brij 35	29	20.4
	Brij 35 + 15% ethanol	14	15.4
Toluene	SDS	240	140
	SDS + 5% methanol	200	124
	SDS + 3% propanol	128	86
	SDS + 10% propanol	114	55
	CTAB	390	190
	CTAB + 5% methanol	240	125

The ϕP_{WS} value are only suggestive since different C18 columns were used. Data from [25].

Figure 6.4 shows the efficiency enhancement for benzene and EAQ obtained with 5% v/v alcohol additions to a 0.285 M SDS micellar phase. The stationary phase is a classical C18 phase ($d_p = 5\mu m$). The X-axis is the $\log P_{WM}$ value of the alcohol, a parameter almost proportional to the alcohol carbon number [27]. In pure SDS solution, the benzene and EAQ efficiencies were respectively 1400 and 49 plates (HETP, 14 d_p and 410 d_p). The maximum efficiencies, 3400 and 1100 plates (5.5 d_p and 18 d_p),

respectively, were obtained with 5% v/v pentanol. The benzene efficiency was more than doubled and the EAQ lipophilic solute was multiplied by a factor 22 (Figure 6.4). The EAQ k retention factor was reduced by 82%, from 45 in pure SDS phase to 8 with 5% v/v pentanol [25]. The maximum efficiency increase for benzene was obtained for butanol and pentanol. The EAQ efficiency seems identical with the addition of pentanol and hexanol (Figure 6.4), but the hexanol efficiency value was obtained with a 0.5 mL/min flow rate (instead of 1 mL/min) due to the high viscosity of the SDS-hexanol phase. With this lower flow rate, hexanol produces an inferior efficiency compared to the 5% v/v pentanol addition. Pentanol seems to be the best alcohol for MLC efficiency enhancement with SDS solutions.

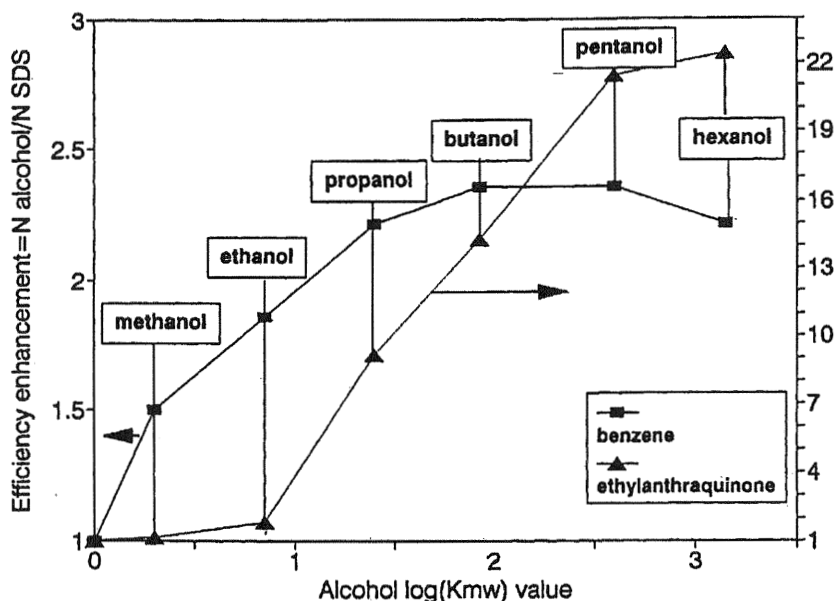


Figure 6.4 Efficiency enhancement obtained with 5% v/v alcohol addition to a 0.285 M SDS phase. Column 10 cm, 4.6 mm i.d., 5 μ m C18 Astec (Whippany, NJ), flow rate 1 mL/min (0.5 mL/min for hexanol), 23°C. Reference efficiency: benzene 1400 plates (left Y-axis), EAQ 49 plates (right Y-axis) in pure SDS micellar phase. Reprinted from [25] with permission of Elsevier.

IV.2. *Effect of Alcohols in MLC*

The main effect of alcohol addition to a micellar phase is the increase of elution strength as reviewed in Chapters 7 and 8 and modeled by the *MICHRON* software (see Appendix I and attached CD-ROM). The decrease of the P values observed for the apolar compounds (Table 6.4) can be generalized to any solute. These effects are related to thermodynamics. The kinetics of the chromatographic process is also affected by the addition of alcohols to the micellar phases.

a) Effect of Alcohols on Micellar Solutions

As briefly exposed in Chapter 2, the addition of medium chain alcohols to micellar solutions change the micelle structure. It was demonstrated that there are two relaxation times in the kinetics of micelle formation-destruction: 1) A fast relaxation time, in the low nanosecond range, corresponding to the exchange rate of a surfactant molecule with the micelle. 2) A slower relaxation time, in the microsecond range, was linked to the average lifetime of a micelle [28]. Methanol and ethanol at low concentrations do not partition significantly with the SDS micelles. Both alcohols do not affect notably the two micelle relaxation times. The C3-C7 alcohols were found to increase the kinetics of the exchange rate of an SDS surfactant or alcohol molecule and to decrease the two relaxation times [29]. The formation-destruction of the SDS micelles is highly accelerated by these alcohols. The alcohol molecules incorporate in the micelles with their polar hydroxyl groups in the Stern layer and their alkyl chain in the micelle cores. This moves the ionic sulfate groups further away [30]. The charge density of the micellar interface decreases which may facilitate the intermicellar migration of solutes. Similar results were obtained with the cationic CTAB surfactant [31]. Low concentrations of C3-C6 alcohols increased the intermicellar migration of a dye solute by several orders of magnitude [32].

For MLC, it is necessary to summarize the vast amount of work done on the effects of alcohols on the ionic micelle structure distinguishing two points: (i) low amounts ($\leq 5\%$ v/v) of methanol and/or ethanol have minimum effect, (ii) the ratio of the alkyl chain length of the alcohol over that of the surfactant, $C_{n_{OH}}/C_{n_{surf}}$, can be used as a reference parameter. If the ratio $C_{n_{OH}}/C_{n_{surf}}$ is lower than 1/3, e.g., propanol and butanol with SDS micelles,

or propanol, butanol and pentanol with CTAB micelles, the alcohol increases the kinetics of both the formation-destruction of the micelle and the exchange rate molecule-micelle. If $C_{n_{OH}}/C_{n_{surf}}$ is higher than 1/3, the concentration of the alcohol should be compared to the surfactant concentration. Low amounts of alcohol increase the kinetics of the micellar exchanges which is good for MLC efficiency. Significant alcohol concentrations disorganize the micelle structure which is unfavorable for MLC efficiency. At a constant surfactant concentration, the maximum beneficial alcohol concentration decreases as the $C_{n_{OH}}/C_{n_{surf}}$ ratio increases.

Table 6.5 Alcohol Effect on Surfactant Adsorption

Mobile phase	Adsorbed amount ($\mu\text{mol}/\text{m}^2$)
SDS 0.05 M	4.6
+ 5% v/v methanol	4.4
+ 3% v/v propanol	3.0
+ 2% v/v pentanol	3.1
CTAB 0.02 M	4.5
+ 5% v/v methanol	4.4
+ 3% v/v propanol	3.2
+ 2% v/v pentanol	2.2

Column ODS Hypersil, C18, 5 μm .

Data from [7].

b) Effect of Alcohols on Stationary Phases

Alcohols and surfactant molecules compete for adsorption on the stationary phase. Alcohol addition reduces the amount of adsorbed surfactant [19, 20, 23]. Table 6.5 lists the amounts of adsorbed surfactant when micellar mobile phases with or without alcohol was used [7]. The surfactant desorption is dependent on the alcohol chain length [7, 19]. Surfactant desorption is linked to a σ_f reduction that should decrease the HETP (eq. 6.6). The kinetics of the surfactant adsorption-desorption process is enhanced by alcohol addition [5, 7, 21]. Spectroscopic studies have shown that short chain alcohols (C1-C3) wet a monomeric C18 silica bonded layer without changing its organization. Oppositely, long chain alcohols (C7-C10)

interpenetrate with the C18 chains [33]. These alcohols decrease the surface tension and viscosity of the C18 bonded layer in minute amounts [33].

c) Which Alcohol Is Best and How Much to Add?

The 1983 work of the Dorsey research group established the use of 3% v/v 1-propanol in micellar phases to reduce the MLC efficiency problem [3]. It is now confirmed that the addition of alcohol to micellar phases: (i) increases the rate of the solute mass-transfer between the micelles and the aqueous phase by increasing the solute micelle exit rate constant, (ii) increases the solute mass transfer kinetics between the stationary phase and the aqueous phase by decreasing the stationary phase viscosity and the amount of adsorbed surfactant. The problem of alcohol addition to micellar phases is that kinetics enhancements cannot be dissociated from thermodynamics changes. The efficiencies increase and the retention times decrease. A hybrid alcohol-micelle mobile phase has necessarily a higher solvent strength than a purely aqueous phase [34]. It was shown that alcohols were changing the micelles and the stationary phase in a comparable manner [26] as noted on the P_{WS} and P_{WM} parallel variations in Table 6.4.

As shown by Figure 6.4 the maximum efficiency increase for benzene was obtained for butanol and pentanol that have Cn_{OH}/Cn_{surf} parameter of 1/3 and 0.42, respectively. 5% v/v hexanol seems to be too much with a Cn_{OH}/Cn_{surf} parameter of 1/2, the benzene efficiency decreases; the reason may be that the SDS micelles are disorganized. Pentanol seems to be the best alcohol with SDS solutions.

A limiting parameter for the choice of alcohol is viscosity. Most hybrid micellar phases obtained with alcohol addition have a higher viscosity than the corresponding micellar phase without alcohol. Viscosity increases dramatically with the alcohol chain length. The viscosity of the 0.285 M SDS phase was 1.65 cP producing a 95 kg/cm² (9.5 MPa or 1400 p.s.i.) pressure at 1 mL/min with a 10 cm column. The viscosity of the 5% v/v pentanol SDS phase was 2.32 cP producing a 127 kg/cm² (12.7 MPa or 1800 p.s.i.) pressure [25]. The viscosity of the 5% hexanol SDS phase was 11.8 cP which precluded its use at 1 mL/min. 0.5 mL/min flow rate was used. This has likely produced overestimated efficiencies for the hexanol points of Figure 6.4.

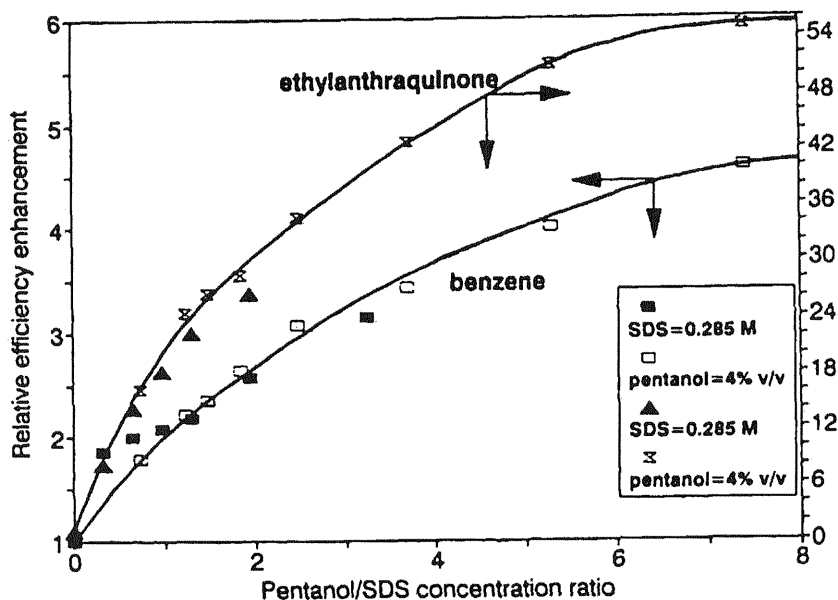


Figure 6.5 Efficiency enhancement versus the pentanol to SDS concentration ratio. Same experimental conditions and reference efficiencies as in Figure 6.4. Reprinted from [25] with permission of Elsevier.

Is 5% v/v the right amount for alcohol addition? Considering that large amounts of alcohols with a Cn_{OH}/Cn_{surf} ratio higher than 1/3 can disorganize the micelle structure [35], the alcohol to surfactant ratio is studied. Figure 6.5 shows the efficiency enhancement obtained for benzene and EAQ plotted versus the pentanol over SDS concentration ratio. Two sets of experiments were done: (i) constant SDS concentration (0.285 M) and increasing pentanol additions, and (ii) 4% v/v pentanol and increasing SDS concentrations [25]. The efficiencies obtained are clearly linked to the pentanol/SDS ratio. The efficiency enhancement reaches a plateau for concentration ratios higher than 6. Similar efficiency enhancements were obtained with the cationic CTAC surfactant, an ODS (C18) column and acetonitrile (ACN) as the organic additive [20]. A plateau at a three fold

efficiency enhancement was obtained for ACN/CTAC concentration ratio higher than 12. Although these results were obtained for only two ionic surfactants and C18 columns, it seems possible to extend them to other micellar mobile phases and bonded silica stationary phases. The alcohol to surfactant ratio acts on both solute exchange rates: micelle-aqueous phase exchanges and stationary phase-mobile phase exchanges. It is directly related to the number of alcohol molecules per micelle. Also, this ratio dictates the total amount of surfactant surface coverage and fluidity of the composite organic layer of the stationary phase [7, 25].

In conclusion, the best amount of alcohol to add depends on the surfactant concentration used. It means again that efficiency enhancements cannot be dissociated from micellar solvent strength.

IV.3. Other Routes for Efficiency Enhancements

Alcohol addition is the most widely used solution to improve efficiency in MLC. Knowing that the inferior efficiency is due to a slow solute exchange rate between micelles and the bulk aqueous solution and/or the surfactant covered stationary phase, two other routes are possible to enhance these exchanges: (i) to raise the temperature and (ii) to decrease the flow rate.

a) Effects of Temperature

Figure 6.6 shows the effect of temperature on the experimental efficiency of anthracene [5] and acetophenone [22] eluted with pure micellar solutions. The efficiency of acetophenone on a C18 column was multiplied by 12 when the temperature was raised from 25°C to 73°C [22]. A threefold enhancement was obtained for anthracene [5]. The retention factors were decreased by temperature. The magnitude of the k decrease is far less important than what was observed with alcohol additions [34]. The k decrease with temperature are predicted by the Van't Hoff equation (eq. 6.4). The equilibrium enthalpies and entropies of the retention process were measured in MLC for various surfactants [36]. These studies showed that the micellar retention process is essentially governed by entropic effects [36].

Enthalpy-entropy compensation studies are useful in the investigation of separation mechanisms. They were used in the study of the retention

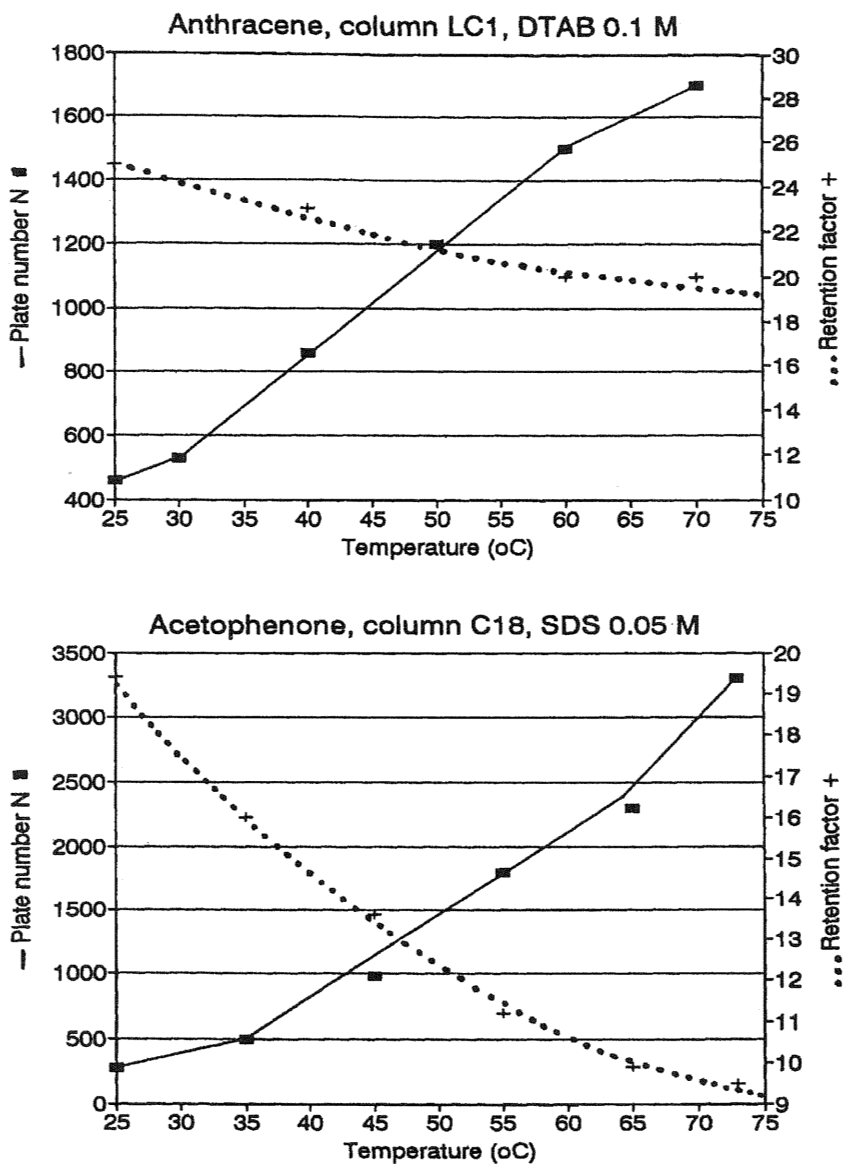


Figure 6.6 Combined changes produced by temperature raises on efficiency (full lines) and retention factor (dotted lines) of Anthracene (top) and Acetophenone (bottom) (Data from [5] and [22]).

mechanisms by C18 phases [37] and the study of the mechanism of chiral separations with cyclodextrin bonded phases in GC [38]. The enthalpy change, ΔH_{WM} , of the P_{WM} values of 6 solutes (the more lipophilic one was naphthalene) were determined in pure SDS micellar phase and 3% and 10% v/v propanol modified SDS phases [39]. The plot of the $\ln k$ value of each solute versus the corresponding ΔH_{WM} value should produce straight lines with similar slopes if the separation mechanisms are similar [37-39]. Such studies showed that the alcohol addition altered the micellar retention mechanism. Propanol altered the stationary phase as well as the micellar phase [39].

The solubility of silica in hydro-organic phases is enhanced by the rise in temperature. The rate of the silica solubilization depends on the pH and temperature of the mobile phase and also on the bonding quality of the stationary phase [40]. Silica solubility may become a serious problem when working at temperature higher than 50°C [5, 22, 39]. Column life is shortened.

b) Flow Rate

Considering the Knox plots, an obvious way to improve efficiency is to work at the optimum flow rate. Table 6.2 lists the optimum flow rates for different mobile phases. The strength of the pure micellar phases is much lower than that of hydro-organic phases [34]. 0.3 mL/min or lower flow rates will produce rebutting retention times. Table 6.2 shows that both alcohol addition and, especially, a rise in temperature increase the optimum flow rates. It was shown that both these remediation methods also increase the solvent strength, decreasing the retention times. If time is not a priority, it should never be forgotten that a decrease of the flow rate can easily further enhance the micellar efficiency and consequently the chromatographic resolution.

c) Classical Efficiency Enhancements

Column overload and silanol interaction may broaden the chromatographic bands with or without micelles in the mobile phase. In case of a peak deformation due to a stationary phase overload, the decrease of the injected

amount will enhance the peak shape. In the case of peak tailing due to silanol-amine interactions, it was shown that the peak shapes greatly improved when the mobile phase pH was decreased to below 5.5 [25]. The observed efficiency increase was attributed to protonation of the silanol groups. Triethylamine is classically added to reversed-phase mobile phases to bind the silanol groups. This reduces the tailing of basic compounds which is not due to the micellar phase. The peak of propranolol in a Brij 35 (nonionic surfactant) mobile phase was greatly improved and its asymmetry decreased by a small addition of triethylamine to the micellar mobile phase [41]. Higher addition of triethylamine produced a decrease of the propranolol retention factor with a slight further amelioration of peak shape and efficiency. This effect is probably due to an organic modifier effect similar to the effect described for alcohols.

V. CONCLUSION

To summarize, the efficiency loss observed in MLC using purely aqueous micellar mobile phases is due to three causes: (i) a slow solute transfer from the aqueous to the micellar phase, (ii) a slow transfer from the stationary phase to the aqueous phase and (iii) a change of the flow pattern in the column, a change due to surfactant adsorption that changes the porosity and surface of the stationary phase.

MLC efficiency can be enhanced: (i) by reducing the mobile phase flow rate to work closer to the optimum of the Knox plot, (ii) by increasing the temperature which decreases the viscosities, increases the rate constants and decreases the amount of adsorbed surfactant, (iii) especially by adding an organic modifier such as an alcohol whose alkyl chain has a length such as the ratio Cn_{OH}/Cn_{surf} close to 1/3. The amount of added alcohol should be increased if the surfactant concentration is increased to keep constant the alcohol to surfactant ratio.

Kinetics and thermodynamics are intimately linked. If flow rate reduction is excluded, all the other efficiency remediation methods produce thinner peaks eluting earlier. Then, the resolution factor may or may not be improved.

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7

Elution Strength and Selectivity with Micellar Systems

I. INTRODUCTION

I.1. Micellar Liquid Chromatography vs. Conventional Reversed-Phase Liquid Chromatography

Micellar Liquid Chromatography (MLC) can be used for separations commonly accomplished by ion exchange, normal phase and/or Reversed-Phase Liquid Chromatography (RPLC), by only selecting an appropriate combination of surfactant type and bonded packing. From the early publications, micelles were indeed thought to replace organic modifiers in RPLC and, in fact, many attempts have been made to demonstrate the unique advantages of MLC over aqueous-organic RPLC.

Despite the capabilities of MLC, the technique has not however received enough attention in solving the "real world" problems in analytical laboratories, for two apparent reasons. First, RPLC with aqueous-organic eluents is a powerful and popular technique that would be very difficult for any other chromatographic method to replace. A reasonable argument is needed for an alternative technique. Second, the two techniques (MLC and conventional RPLC) have been often compared in an area in which MLC has clear disadvantages, that is, for the separation of uncharged and hydrophobic compounds. MLC is a poor choice in this respect from the three important aspects of efficiency, elution strength and selectivity.

As shown in the previous chapter, column efficiency in MLC is very often inferior to that achieved with aqueous-organic systems, and deteriorates as the hydrophobicity of solutes increases. Also, pure micellar eluents are generally weak: long analysis times are thus observed for hydrophobic solutes. Separation selectivity for these compounds is small,

as compared to aqueous-organic mobile phases at similar elution strength, due to the similar environments of mobile and stationary phases in MLC. The problem with the efficiency, elution strength and selectivity, can be improved by the addition of a small concentration of an organic modifier to the micellar mobile phase; however, even this system may not be as suitable as aqueous-organic systems for the separation of hydrophobic compounds.

On the other hand, it is impossible for any homogeneous aqueous-organic or mixed organic mobile phase to achieve the range of interactions found in MLC. Owing to the amphiphilic nature of surfactants, solutes can associate with micelles through a combination of electrostatic, hydrophobic and steric interactions. For this reason, micellar eluents are compatible with ionic and water-insoluble compounds. The main strength of MLC lies precisely in the capability of performing and controlling the separation of mixtures of cationic, anionic, and uncharged polar and nonpolar solutes, with isocratic elution. In this respect, micellar mobile phases offer a clear advantage in terms of chromatographic selectivity over conventional aqueous-organic eluents.

I.2. Variables that Can Be Controlled to Enhance the Elution Strength and Selectivity

It appears that MLC can offer several mechanisms for manipulating retention times and, as a consequence, controlling the selectivity. Parameters such as the type and concentration of surfactant and organic solvent, pH, ionic strength and temperature, can be used for this purpose.

With the wide variety of surfactants available, including ionic (anionic or cationic), nonionic and zwitterionic, many unique separations and applications can be developed. Because organic modifiers do not decrease the equilibria equally for all solutes, they can also change the selectivity of the mobile phase. Moreover, with electrostatic interactions playing a major role in the selectivity, it is important to specify the pH of the mobile phase. For instance, the measured pH of a 0.1 M sodium dodecyl sulfate (SDS) solution is 6.1 and that of 0.5 M cetyltrimethylammonium bromide (CTAB) is 4.2. These pH values can obviously be changed by appropriate buffers, so that some solutes can be present in ionized form.

Some examples will be given in this chapter to show the interest and consequences of manipulating all these variables. The importance of pH in controlling the selectivity in the separation of mixtures of solutes experiencing protonation equilibria is noteworthy. However, it will not be discussed in this chapter, but in Chapter 8, where some new knowledge on the combination of surfactant-mediated equilibria with protonation equilibria is introduced.

II. ELUTION ORDER REVERSALS

Interesting selectivities were reported from the early publications of MLC, mostly in the form of elution reversals. Peak crossing is often considered proof of enhanced selectivity. However, although elution order reversals are indicative of a unique separation mechanism in MLC, peak crossing in a complex mixture does not necessarily mean an improvement in the overall separation.

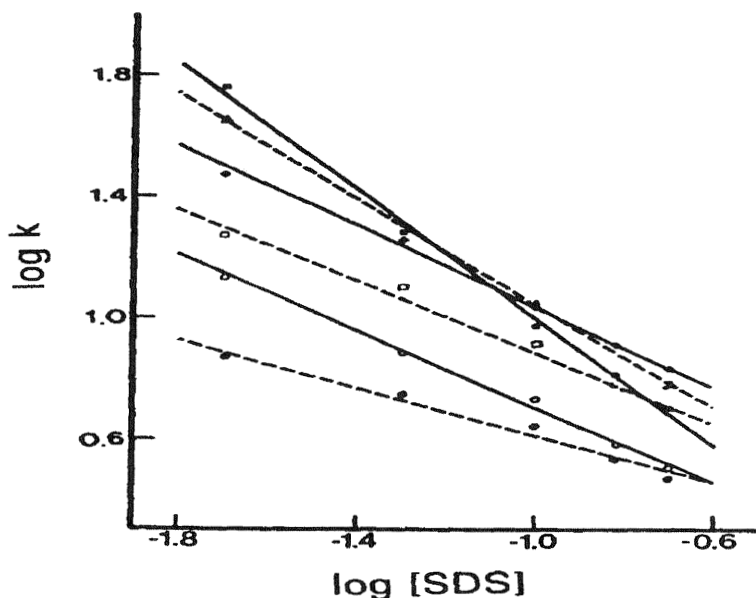


Figure 7.1 Effect of sodium dodecyl sulfate on the retention of: phenol (●), *p*-nitroaniline (○), benzene (□), nitrobenzene (◆), toluene (▲), and 2-naphthol (■). Reprinted from Ref. 1 with permission of the American Chemical Society

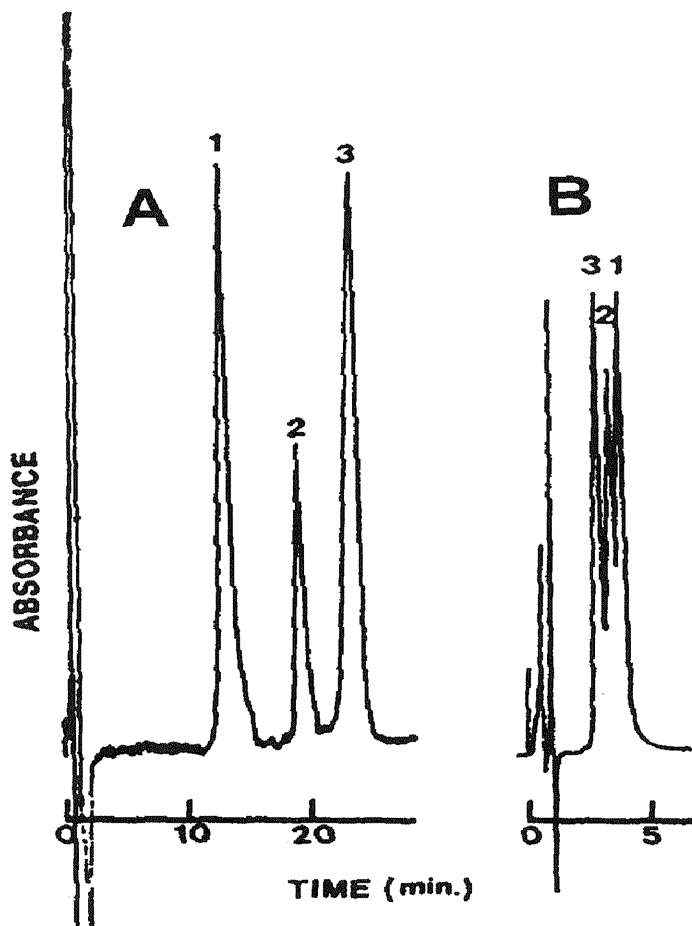


Figure 7.2 Chromatograms of: (1) nitrobenzene, (2) toluene and (3) 2-naphthol, eluted with 0.02 M SDS (A), and 0.20 M SDS (B). Reprinted from Ref. 1 with permission of the American Chemical Society.

When the logarithm of the retention factor ($\log k$) is plotted against the logarithm of surfactant concentration, as in Fig. 7.1, one important feature of MLC becomes apparent. The linear plots are not parallel, but

intersect one another. Convergence, divergence and reversal of the peaks can occur as a result of an increase in micelle concentration. Thus, not only the retention factor but also the selectivity coefficient, α (defined as the ratio between the retention factors of two solutes, k_a/k_b , where α is larger than 1), are changing. In Fig. 7.1, the plots for nitrobenzene, toluene and 2-naphthol cross. The consequences of these crossings are illustrated in Fig. 7.2, where the elution order is nitrobenzene, toluene, 2-naphthol, with 0.02 M SDS, while it is reversed with 0.20 M SDS. The separation can therefore be controlled by the simple measure of changing the concentration of surfactant.

The Armstrong equation (Chapter 5) describes the retention in MLC as [2]:

$$k = \frac{\phi P_{ws}}{1 + v(P_{wm} - 1)[M]} \quad (7.1)$$

where $\phi = V_s/V_o$ is the phase ratio (V_s is the volume of the active surface of the stationary phase and V_o the column dead volume), v the partial specific volume of the monomers of surfactant, P_{ws} and P_{wm} the partition coefficients between water and stationary phase, and water and micelles, respectively (remember $K_{AM} = v(P_{wm} - 1)$ is the solute-micelle binding constant), and $[M]$ the micelle concentration.

In Table 7.1, several solutes are listed in their elution order by the use of 0.02 M SDS. At this low micelle concentration, the system resembles RPLC and P_{ws} controls the retention, as seen by the increased retention times with increasing P_{ws} values. As the concentration of surfactant is increased, the importance of K_{AM} increases, due to the larger number of micelles present in the mobile phase. The larger the value of K_{AM} , the greater the effect of the increasing concentration, as seen by the steeper slopes in the log-log plots for the more retained compounds (Fig. 7.1). The effect of P_{ws} on retention is independent of SDS micelle concentration, since the amount of surfactant adsorbed on the stationary phase remains essentially constant after equilibration, once the concentration is above the critical micellar concentration (cmc).

Table 7.1 Water-Stationary Phase and Water-Micelle Equilibrium Constants for Sodium Dodecyl Sulfate Micellar Chromatographic System [1]

Compound	SDS		
	P_{WS}	P_{WM}	K_{AM} (L/mol)
Phenol	5.0	160	39
<i>p</i> -Nitrophenol	8.7	314	77
<i>p</i> -Nitroaniline	9.4	342	84
Benzene	13.8	314	77
Nitrobenzene	22.4	367	90
Toluene	41.3	875	215
2-Naphthol	59.5	1600	393

P_{WS} and P_{WM} are dimensionless constants, K_{AM} is in L/mol because the molar volume value for SDS used was $v = 0.246$ L/mol.

2-Naphthol has larger K_{AM} value than the other solutes and, consequently, the steepest slope. As the concentration of SDS increases, the retention of this compound decreases faster than the retention of nitrobenzene or toluene, so that elution reversals occur. Since K_{AM} for toluene is also higher than for nitrobenzene, complete reversal of elution order takes place for 2-naphthol, toluene and nitrobenzene, going from 0.02 M SDS to 0.20 M SDS. While K_{AM} for 2-naphthol is much higher than for the early eluting solutes such as phenol, the difference in the values of P_{WS} is so large that very high SDS concentrations would be needed for these two compounds to show a change in elution order.

III. THE CHOICE OF SURFACTANT AS A MEANS TO CONTROL SELECTIVITY

III.1. Charge of the Surfactant

A mode of selectivity can be employed via the selection of ionic surfactant (e.g., anionic or cationic), since polar solutes can interact electrostatically,

as well as hydrophobically with these surfactants. As previously stated, there is considerable adsorption of surfactant onto a nonpolar stationary phase, thereby providing electrostatic interactions of ionizable solutes with the stationary phase, as well as with the charged micelles. With an appropriate surfactant, mixtures of polar and nonpolar solutes can be resolved adequately. It is often possible to perform the separation of a greater number of compounds when working with mobile phases of low surfactant concentration, due to the higher retentions. However, the analysis duration is longer.

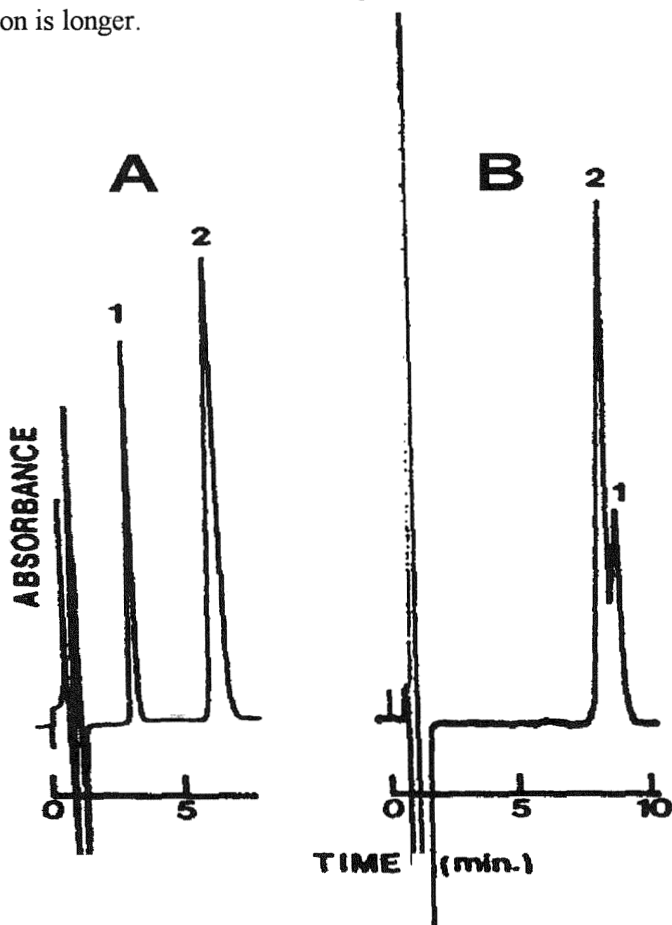


Figure 7.3 Chromatograms of: (1) phenol, and (2) benzene, eluted with 0.05 M SDS (A), and 0.05 M DTAB (B). Reprinted from Ref. 1 with permission of the American Chemical Society.

One interesting example of the selection of the surfactant charge was provided by the comparison of two surfactants, one anionic (SDS), the other cationic (dodecyl trimethylammonium bromide, DTAB), in the separation of a mixture of aromatic compounds. These surfactants form comparable micelles, differing essentially only in the nature of the polar end group. Both contain a linear C-12 chain as their primary hydrophobic portion and have similar cmc of 8.1×10^{-3} M and 1.6×10^{-2} M, respectively [1]. Figures 7.3 and 7.4 show that dissociated phenol and benzene are not well resolved with DTAB, but are completely resolved with SDS. In contrast, *p*-nitroaniline and *p*-nitrophenol are not separated with SDS, but are well resolved with DTAB micellar phases. Also, benzylamine (not shown), which is unretained with DTAB, interacts strongly with SDS and is retained ($k = 21$ with 0.10 M SDS).

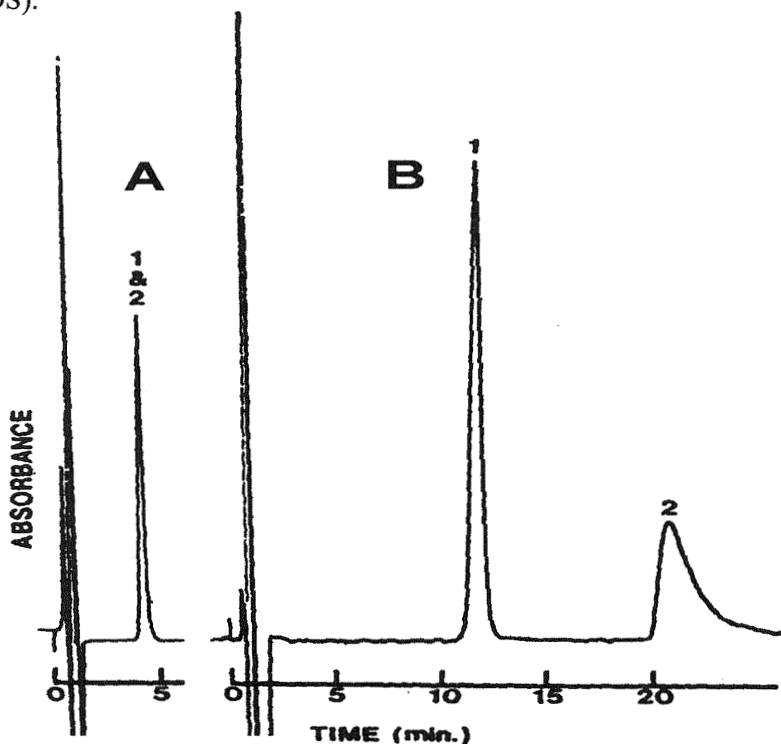


Figure 7.4 Chromatograms of: (1) *p*-nitroaniline, and (2) *p*-nitrophenol, eluted with 0.05 M SDS (A), and 0.05 M DTAB (B). Reprinted from Ref. 1 with permission of the American Chemical Society.

III.2. Interactions with the Stationary Phase

The ability of micelles to solubilize and to interact selectively with solute molecules is believed to be the basis of separation in MLC. However, surfactant molecules readily adsorb on bonded stationary phases, such as C18 or C8, and the modifications produced can have profound implications with regard to retention and selectivity. Solute-stationary phase interactions in MLC are thus very important. Perhaps some of the reported differences in selectivity, between MLC and RPLC with aqueous-organic mobile phases, are due in some measure to the modification of the stationary phase by adsorbed surfactant.

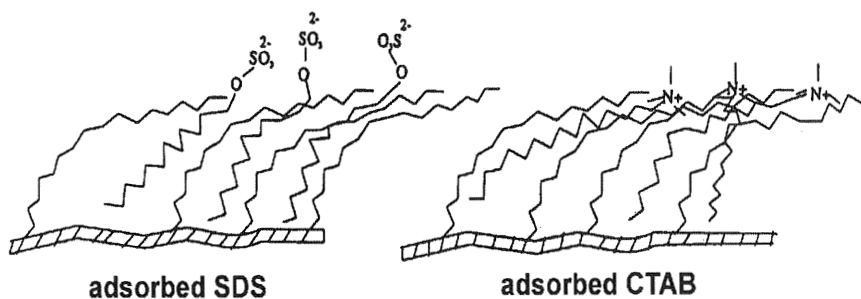


Figure 7.5 Structures of SDS- and CTAB-modified C18. Reprinted from Ref. 3 with permission of the American Chemical Society.

The role of the surfactant-modified stationary phase in an MLC separation process was investigated by employing a set of six vanillin compounds (vanillin, o-vanillin, isovanillin, ethylvanillin, vanillic acid and coumarin), as retention probes [3]. Differences in selectivity between SDS and CTAB micellar mobile phases were found to be due to the differing nature of the surfactant bonded-phase association. For SDS, the hydrophobic alkyl tail is associated with the bonded alkyl phase, with the sulfate head group projected away from the surface (Fig. 7.5). Adsorption of SDS would lead therefore to the formation of a hydrophilic layer on a C18 phase. This simplified model was also suggested by Armstrong et al. [4] to explain poor stationary phase mass transfer in MLC. According to these authors, a hydrophobic solute has to traverse the hydrophilic boundary layer

formed by sulfate head groups, ions and associated water, in order to gain access to or leave the stationary phase. The boundary layer can obstruct mass transfer between bulk solvent and bonded phase.

For CTAB adsorbed on C18, the nitrogen head group is probably oriented closer to the silica surface due to hydrophobic interaction between the *N*-methyl groups of the surfactant and the bonded phase (Fig. 7.5). The nitrogen head group is thus probably incorporated partially or totally into the bonded phase, giving rise to a modified bulk phase that is significantly denser. Incorporation of CTAB would ensure that much of the hydrophobic character of the original bulk phase is retained.

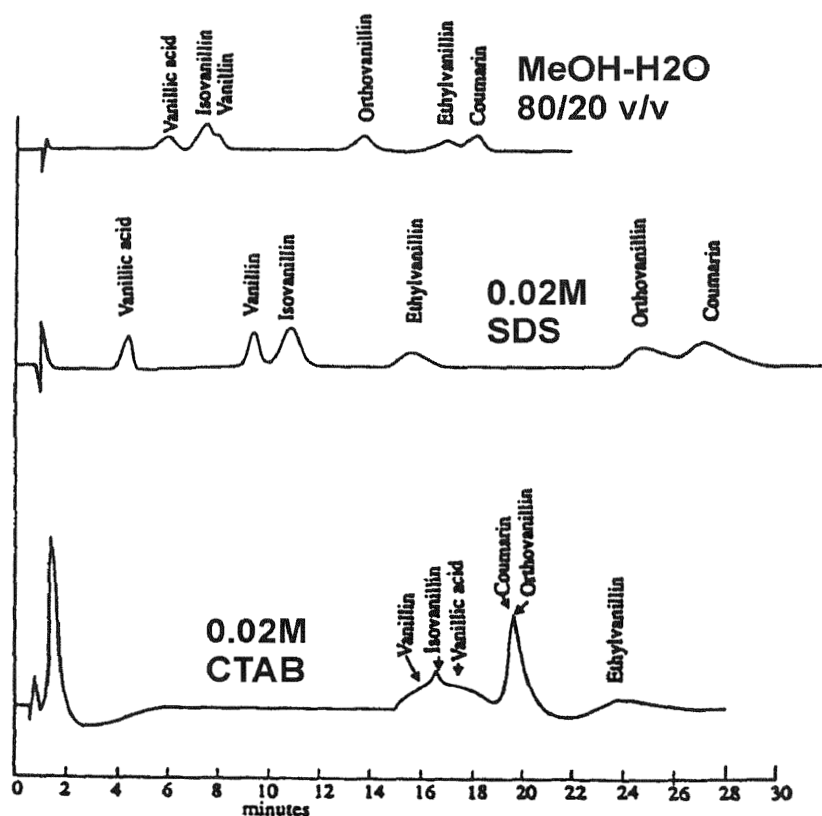


Figure 7.6 Chromatograms of a mixture of vanillin compounds, separated on a C18 column with micellar mobile phases of different nature at pH 3. From top to bottom: methanol-water 20:80 (v/v), 0.02 M SDS and 0.02 M CTAB. From Ref. 3.

Figure 7.6 shows the separation of the mixture of vanillins on a C18 column with the following mobile phases: methanol-water 20:80 (v/v), 0.02 M SDS and 0.02 M CTAB. Several things are apparent from an examination of the chromatograms. First, the test mixture is completely separated by the SDS micellar mobile phase, but not by the aqueous-organic or CTAB mobile phases. Second, the efficiency of the chromatographic process is poor for all three mobile phases, and this implies that the variability in the resolution of the test mixture, among the three mobile

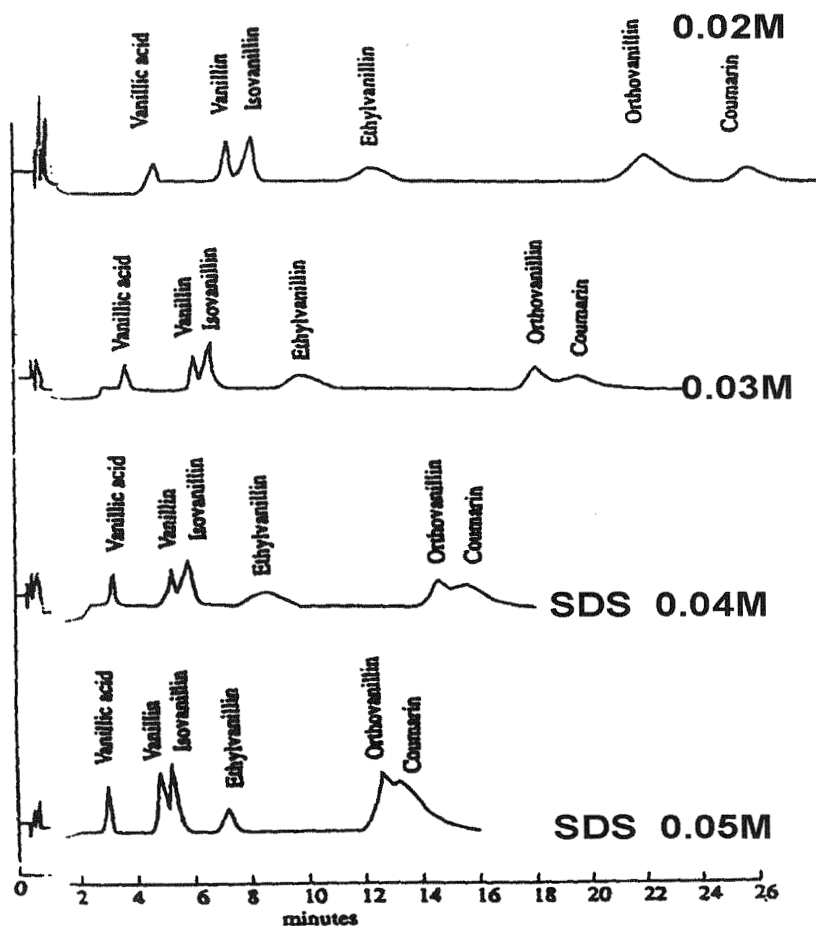


Figure 7.7 Chromatograms of a mixture of vanillin compounds, separated on a C18 column with micellar mobile phases, at pH 3 and varying SDS concentration. Molar concentration of SDS from top to bottom: 0.02, 0.03, 0.04, and 0.05. From Ref. 3.

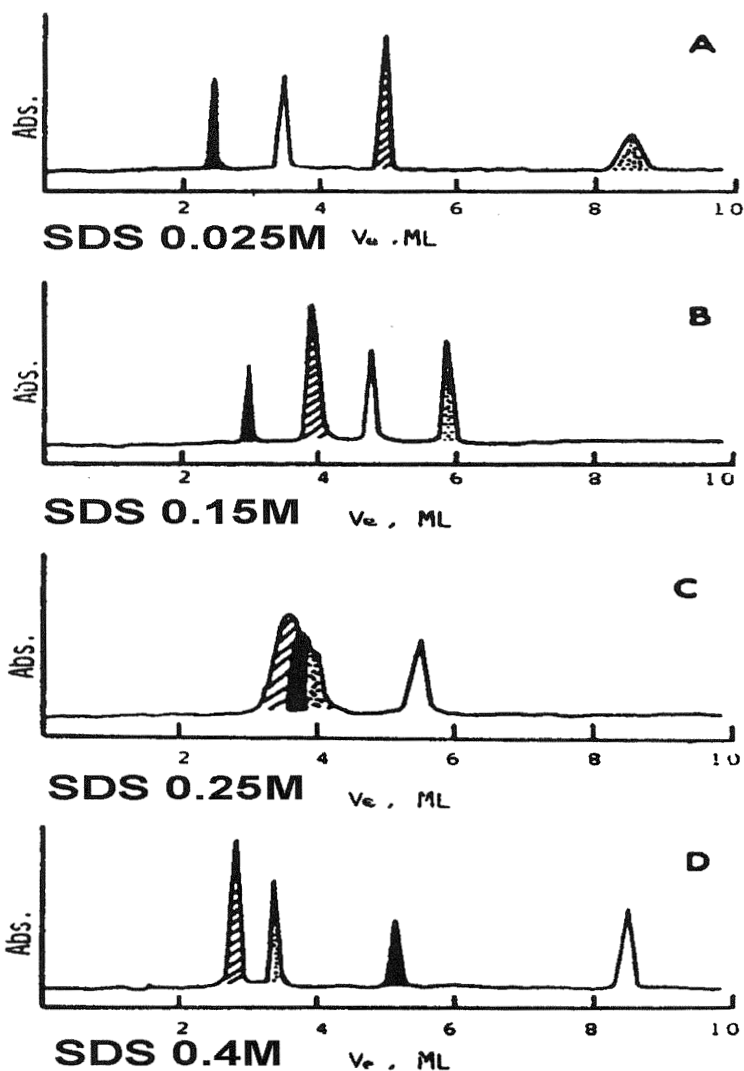


Figure 7.8 Separation of two binding compounds (lined peak, naphthalene; dotted peak, *p*-nitrophenol), and two antibinding compounds (solid peak, 2-naphthol-6-sulfonic acid; open peak, sodium naphthalenesulfonate) with a cyano column and SDS micellar mobile phases. Molar concentration of SDS: (A) 0.025, (B) 0.15, (C) 0.25, and (D) 0.40. From Ref. 5.

phases, is due to differences in selectivity. Third, the retention time of four of the six vanillin compounds is greater with 0.02 M CTAB, which suggests that the interaction between each of these four compounds and the stationary phase is greater with CTAB-modified C18 than with SDS-modified C18. This would explain the superior resolution achieved with SDS for the vanillin compounds, which are also hydrophilic and probably undergo some type of selective hydrogen-bonding interaction with the bonded layer.

Although SDS adsorption enhances the selectivity of the stationary phase toward the vanillin compounds, SDS micelle-solute interactions also contribute to the selectivity of this separation. For example, SDS micelles interact more strongly with vanillin than with isovanillin, as evidenced by the greater K_{AM} binding constant for vanillin, and this interaction is responsible, at least in part, for the baseline resolution of these two compounds. Nevertheless, the successful separation of the vanillin compounds with the 0.02 M SDS mobile phase is primarily due to solute-stationary phase interactions, which is also the reason why the separation of the vanillin test mixture is more favorable at lower SDS concentrations (see Fig. 7.7).

III.3. Separation of Mixtures of Binding and Antibinding Compounds

Some separations may involve mixtures containing binding, nonbinding and antibinding compounds. By choosing the correct micellar mobile phase, it is possible to separate and distinguish between these three classes of compounds. Figure 7.8 illustrates the separation of a mixture containing binding (*i.e.*, naphthalene and *p*-nitrophenol), and antibinding (*i.e.*, 2-naphthol-6-sulfonic acid and sodium naphthalenesulfonate) compounds. In each successive chromatogram (A through D), the concentration of SDS in the mobile phase was increased. When the SDS contents of the mobile phase increased, the retention of naphthalene and *p*-nitrophenol decreased, while 2-naphthol-6-sulfonic acid and sodium naphthalenesulfonate eluted at longer times. Note that optimum resolution was obtained either at high or low SDS concentrations (*i.e.*, chromatograms A and D, respectively), and that peak inversion occurred.

IV. SOLUBILITY LIMIT THEORY AND SELECTIVITY

The solubility limit theory explains the direct transfer of highly hydrophobic solutes from micelles to the stationary phase [6]. The retention factor for these compounds can be expressed as:

$$k = \frac{\phi P_{MS}}{v [M]} \quad (7.2)$$

where P_{MS} is the partition coefficient between micelles and stationary phase (from eq. 7.1 with $P_{MS} = P_{WS}/P_{WM}$ and $P_{WM} \gg 1$).

The implications on selectivity of the direct transfer furnishes a new evidence of the solubility limit theory. The retention mechanism of several hydrophobic compounds (i.e., benzene derivatives, polycyclic aromatic hydrocarbons (PAHs), and dihydropyridines) was studied in SDS and CTAB micellar systems, by comparing experimental selectivity coefficients with those theoretically calculated assuming a direct transfer mechanism [7, 8]. A mathematical expression was derived by using the three-partition equilibria theory, which explains the tendency of selectivity coefficients to the ratio of P_{MS} coefficients of the solutes, when the concentration of surfactant increases. Expressing the equation that relates the retention with the concentration of micelles as a function of P_{MS} and P_{WM} :

$$k = \frac{\phi P_{MS}}{1/P_{WM} + v(1 - 1/P_{WM})[M]} \quad (7.3)$$

the selectivity coefficient for a given pair of compounds (i.e., a and b) will be defined by:

$$\alpha = \frac{P_{MS,a} [1/P_{WM,b} + v(1 - 1/P_{WM,b})[M]]}{P_{MS,b} [1/P_{WM,a} + v(1 - 1/P_{WM,a})[M]]} \quad (7.4)$$

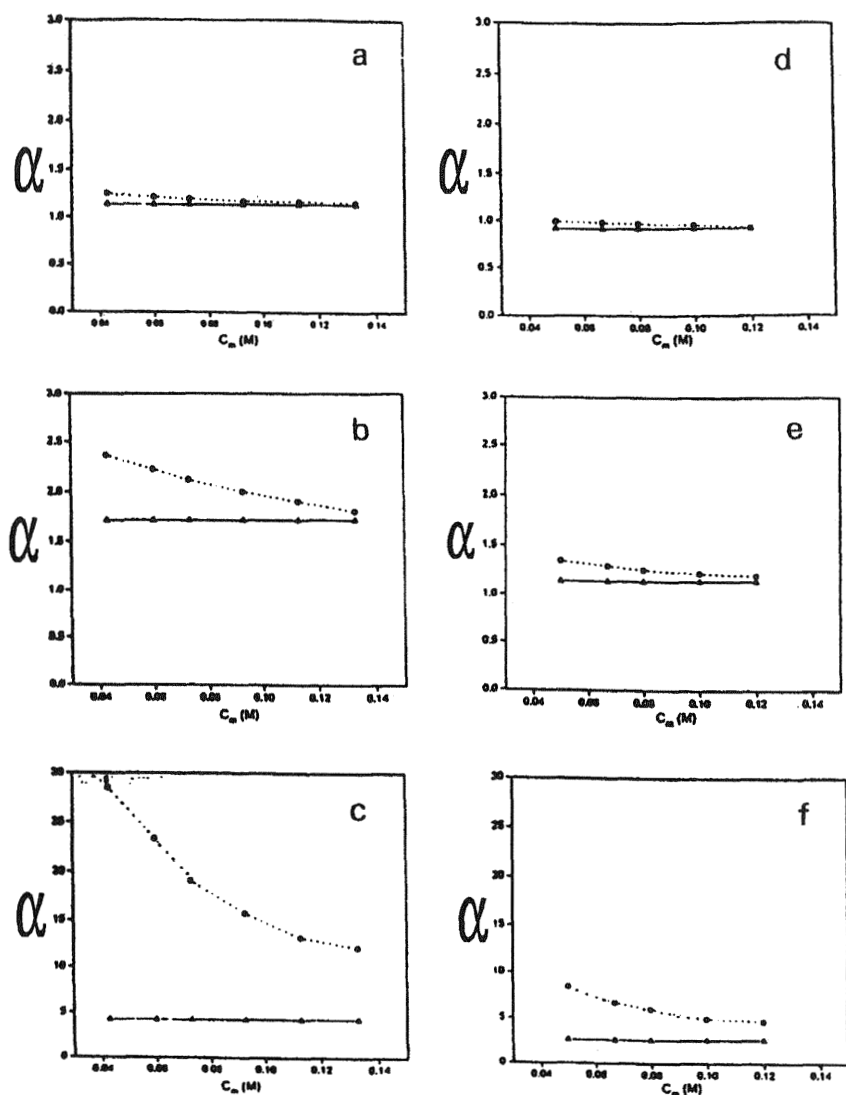


Figure 7.9 Variation in the experimental (O) and theoretical selectivity (▲) coefficients, as a function of micelle concentration, for three pairs of solutes: pyrene-acenaphthene (top), pyrene-toluene (middle), and pyrene-benzamide (bottom), in SDS-5% 1-propanol (a-c) and CTAB-5% 1-butanol (d-f) mobile phases. From Ref. 8.

As the P_{WM} coefficients for hydrophobic compounds are high, it is possible to consider that $(1 - 1/P_{WM}) \approx 1$. Also when $[M]$ is increased, $v[M]$ can be large enough to make $(v[M] + 1/P_{WM}) \approx v[M]$. It finally results with:

$$\alpha = \frac{P_{MS,a}}{P_{MS,b}} \quad (7.5)$$

Figure 7.9 shows the variation of the theoretical (eq. 7.5) and experimental selectivity coefficients (calculated from the retention factors), as a function of micelle concentration, in SDS-5% 1-propanol and CTAB-5% 1-butanol mobile phases, for three pairs of solutes: pyrene-acenaphthene which are both very hydrophobic and for which a direct transfer mechanism can be assumed for any surfactant concentration, pyrene-toluene in which only for pyrene can a direct transfer mechanism be assumed for all surfactant concentrations, and pyrene-benzamide in which benzamide does not experience a direct transfer, except at very high surfactant concentration. When both solutes experience direct transfer, the experimental and theoretical selectivity coefficients are very similar (Fig. 7.9 a, d). It is possible to predict the selectivity coefficient from P_{MS} partition coefficients. In contrast, when one of the two solutes does not experience a direct transfer mechanism, the theoretical and experimental selectivity are different but this difference decreases under the conditions in which the direct transfer is favored (Fig. 7.9 b, c, e, f).

Therefore, for certain solutes, the selectivity coefficient tends toward a value that does not depend on the concentration of surfactant in solution. This tendency is due to a change in the retention mechanism from a three-partition equilibria mechanism to a direct transfer of the solutes, from the micelles to the stationary phase. This change is favored when: (i) the hydrophobic character of the solute increases, (ii) CTAB instead of SDS is used as surfactant, and (iii) the polarity of the aqueous mobile phase is increased. With regard to the surfactant nature, it should be said that the partition coefficients of aromatic compounds are generally greater with CTAB than with SDS. This is due to electrostatic interaction between the positively charged CTAB micelles and the unlocated charge of the aromatic rings. When the polarity of the mobile phase is decreased by adding an

alcohol, the affinity of a hydrophobic solute for the aqueous bulk phase increases, subsequently increasing the difference between the theoretical and experimental coefficients. This difference is minimal when using 1-propanol in the mobile phase, which has a higher polarity than 1-butanol.

V. EFFECT OF ORGANIC MODIFIERS ON THE ELUTION STRENGTH AND SELECTIVITY

Micellar eluents composed of only surfactant are generally weak and suffer from poor efficiency. Although the elution strength can be increased adequately in certain instances by increasing the micelle concentration, the chromatographic efficiency usually deteriorates. Addition of an organic solvent to the micellar eluent may give an adequate elution strength, but can also improve the chromatographic efficiency and lead to selectivity enhancements. All this will have a favorable effect on both resolution and analysis time. The use of an organic modifier is however not always appropriate. Selectivity enhancements might not lead to an improvement in resolution if the retention falls below the optimum k range, as a result of an increase in elution strength. In other instances, the addition of an organic solvent to micellar eluents may have a beneficial effect on retention, but the efficiency may remain low.

The rate of change in retention of different solutes varies with their charge and hydrophobicity, as well as with the nature of surfactant and organic modifier in the mobile phase. The partition coefficients of hydrophobic solutes decrease more than those of hydrophilic solutes, with an increasing concentration of alcohol. Hence, the selectivity is modified. As an example of the influence of the modifier on the separation, Fig. 7.10 shows the chromatograms of a mixture of PAHs eluted with mobile phases containing a fixed concentration of micelles (0.15 M SDS), in the absence of modifier and with different modifiers. As seen, the separation is impossible in the absence of modifier, as the most hydrophobic PAHs appear as very wide overlapping peaks, not well differentiated from the baseline. A mobile phase of 0.15 M SDS-15% 2-propanol gives much better separation with shorter analysis time.

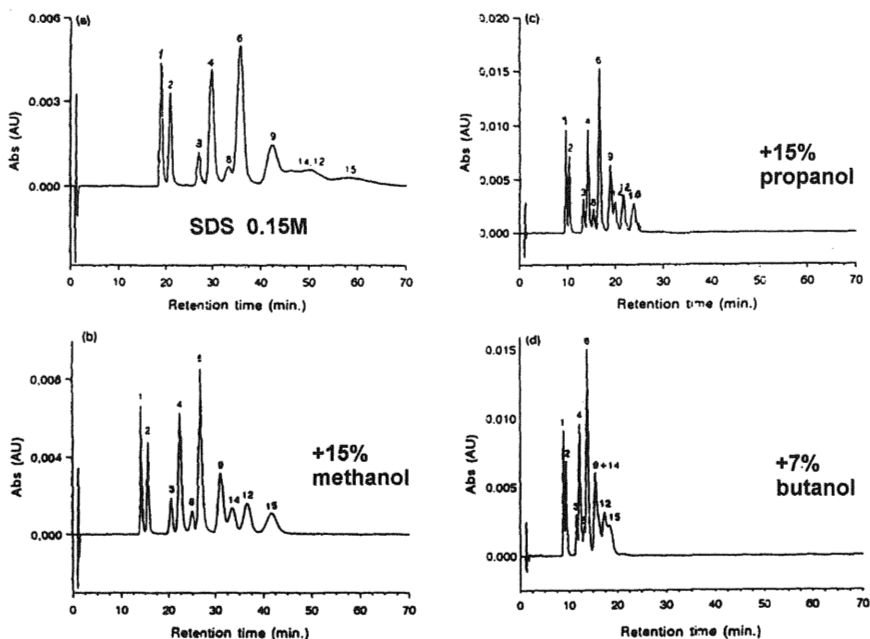


Figure 7.10 Elution of a mixture of ten PAHs with a micellar mobile phase of 0.15 M SDS: (a) without modifier, (b) with 15% methanol, (c) with 15% 2-propanol, and (d) with 7% 1-butanol. PAHs: (1) naphthalene, (2) acenaphthylene, (3) fluorene, (4) anthracene, (6) 9-methylanthracene, (8) pyrene, (9) chrysene, (12) benzo[*a*]pyrene, (14) perylene, and (15) dibenz[*ac*]anthracene. Reprinted from Ref. 9 with permission of Elsevier.

Figure 7.11 illustrates the chromatographic selectivities of 2-propanol, acetonitrile and tetrahydrofuran, in the presence of 0.02 M SDS, for a mixture of seven amino acids and peptides. The volume fraction of organic solvents were adjusted so that the total analysis times of the three mobile phases were approximately the same. The elution order and selectivity of all solutes were similar for the three modifiers, except for the different elution order of peaks 1 and 3 for 2-propanol, as compared to those for acetonitrile and tetrahydrofuran, and poor resolution of peaks 5 and 6 for 2-propanol and acetonitrile. The chromatograms of amino acids and peptides for different concentrations of 2-propanol and 1-butanol, at a fixed micelle concentration (0.08 M SDS) in the hybrid systems, are illustrated in

Figure 7.12. The strengths of both hybrid mobile phases were also adjusted so that the retention of the last peak remained the same. It can be observed that, while all peaks were well separated for 2-propanol, there existed strong overlaps and coelution of peaks 3, 4 and 5 (Fig. 7.12b), and 1-2 and 3-4 (Fig. 7.12d), for 1-butanol.

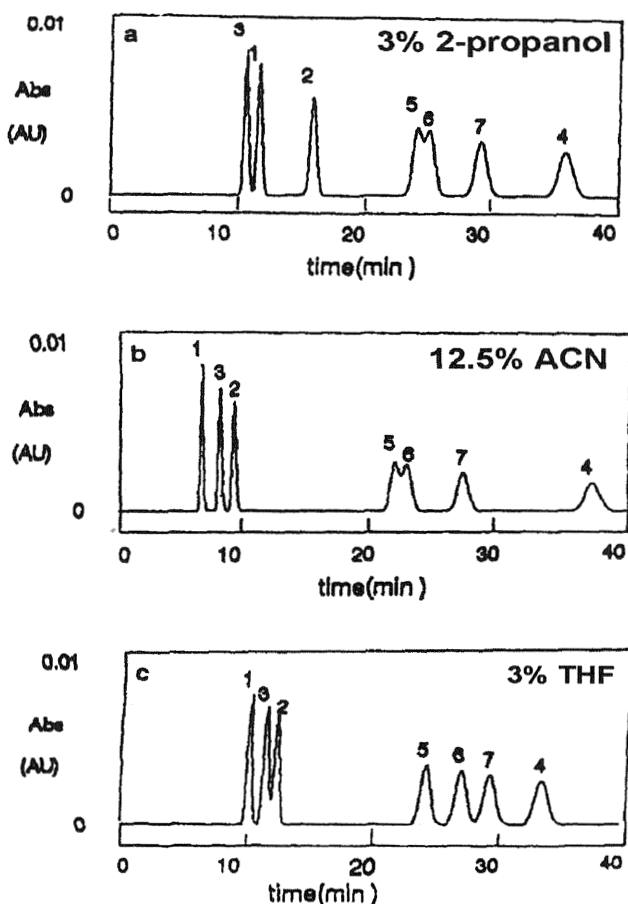


Figure 7.11 Chromatograms of a mixture of amino acids and peptides eluted with 0.02 M SDS mobile phases containing: (a) 3% 2-propanol, (b) 12.5% acetonitrile, and (c) 3% tetrahydrofuran. Compounds: (1) tyrosine, (2) methionine, (3) alanyl-tyrosine, (4) tryptophan, (5) aspartyl-phenylalanine, (6) leucyl-tyrosine, and (7) glycyl-leucyl-tyrosine. Reprinted from Ref. 10 with permission of Elsevier.

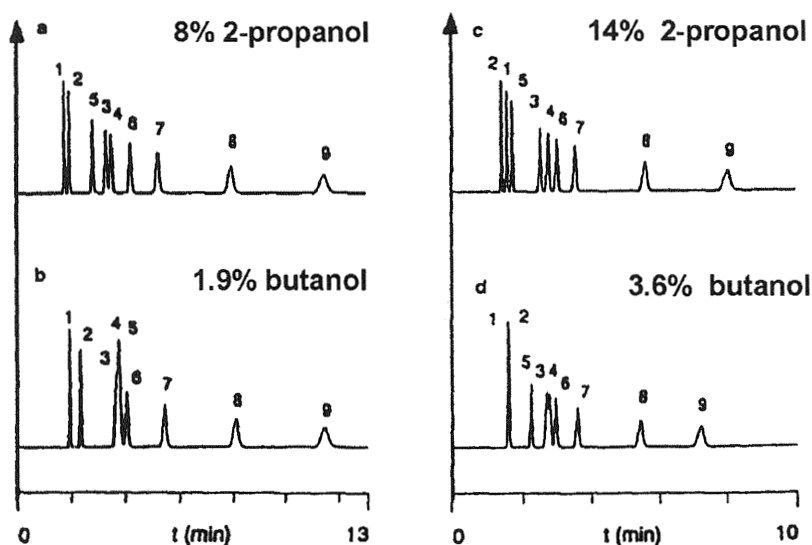


Figure 7.12 Chromatograms of a mixture of amino acids and peptides eluted with 0.08 M SDS and: (a) 8% 2-propanol, (b) 1.9% 1-butanol, (c) 14% 2-propanol, and (d) 3.6% 1-butanol. See Figure 7.11 for compounds 1-7. Other compounds are: (8) leucyl-tri- α -amino acid, and (9) phenylalanyl-phenylalanine. From Ref. 10.

VI. RELATIONSHIP BETWEEN ELUTION STRENGTH AND SELECTIVITY

An extensive investigation was made by Khaledi et al. to better understand the effect of adding organic solvents to micelles, for controlling the retention and selectivity in MLC, and how it compares with aqueous-organic RPLC systems. The retention behavior in MLC can be quite different from that in aqueous-organic RPLC despite the fact that, for both systems, hydrophobic interactions are the main driving force for retention. In effect, addition of an organic solvent to micellar eluents does not create an aqueous-organic system [11, 12]. The retention characteristics of solutes with a ternary micelles-water-organic solvent eluent are similar to those in a pure aqueous

micellar eluent. This means that in the hybrid mobile phases, it is micelles that influence the role of the organic cosolvent in the mobile phase.

VI.1. Measurement of Elution Strength

In RPLC with binary aqueous-organic mobile phases, the relationship between the retention factor (i.e., $\log k$) and the volume fraction of organic modifier (e.g., methanol), ϕ , is often a quadratic equation [13]:

$$\log k = \log k_w + A\phi + B\phi^2 \quad (7.6)$$

where the coefficient A is expected to be negative and B positive, and $\log k_w$ is the logarithm of the retention factor of a solute in pure water, a measure of the interaction of the solute with a given stationary phase. Coefficients A and B are directly related to the elution strength of the organic modifier.

Over a limited range of ϕ values, the relationship between the retention factor and ϕ can be reduced to:

$$\log k = \log k_w - S\phi \quad (7.7)$$

where the slope of the line, S , is the solvent or elution strength parameter, which is generally proportional to the retention and molecular weight of solutes. The linearity of eq. 7.7 deteriorates in the low (less than 20% v/v) and high concentrations (more than 80% v/v) of organic solvents. For two solutes a and b , where $k_b > k_a$, S_b is often larger than S_a . As a result, the selectivity between the two solutes will decrease with an increase in organic modifier concentration.

Khaledi et al. [11] suggested that a similar relationship was valid in MLC with hybrid eluents of micelles-organic modifier:

$$\log k = \log k_O - S_{hyb} \phi \quad (7.8)$$

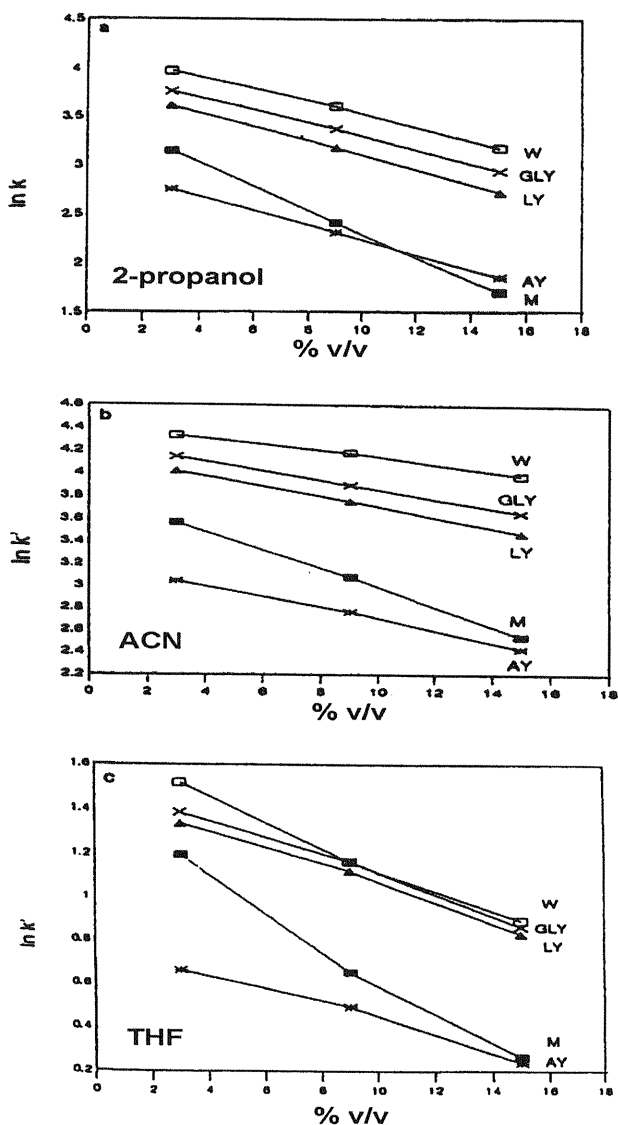


Figure 7.13 Plots of $\log k'$ vs. ϕ for diverse amino acids and peptides eluted with SDS mobile phases containing: (a) 2-propanol, (b) acetonitrile, and (c) tetrahydrofuran. Compounds: tryptophan (W), glycyl-leucyl-tyrosine (GLY), leucyl-tyrosine (LY), alanyl-tyrosine (AY), and methionine (M). Reprinted from Ref. 10 with permission of Elsevier.

where S_{hyb} is the solvent strength parameter in hybrid micellar systems and $\log k_0$ is the retention in pure aqueous micellar eluent (i.e., without organic modifier).

Figure 7.13 shows plots of eq. 7.8, for several amino acids and small peptides, as a function of volume fraction of three organic modifiers: 2-propanol, acetonitrile and tetrahydrofuran, over a 3-15% v/v range. Although the linearity achieved is not good enough, the logarithmic relationship between $\log k$ and ϕ is useful to compare the elution strength for each modifier.

The solvent strength parameters, S and S_{hyb} , represent the sensitivity of solute retention with volume fraction of organic modifier in aqueous-organic and hybrid systems, respectively. Otherwise, the relationship between the slope and the intercept of eqs. 7.7 and 7.8 has a significant effect on chromatographic selectivity. The selectivity between those solutes, whose slopes and intercepts are directly related to one another, will decrease with an increase in organic solvent concentration. In contrast, for cases where there is no direct relationship between the slope and the intercept, the selectivity may increase with organic solvent concentration [10, 14]. In fact, in RPLC with methanol-water mobile phases, linear correlations have been reported for the slope vs. intercept of eq. 7.7, for a large group of compounds [13]. For hybrid eluents in MLC, plots of slope vs. intercept of eq. 7.8 are given in Fig. 7.14. As shown, unlike conventional aqueous-organic eluents in the presence of micelles, no correlation was observed between S_{hyb} and $\log k_0$ for 2-propanol, acetonitrile and tetrahydrofuran-modified micellar eluents. One can then anticipate, therefore, a different selectivity behavior for these organic solvents in the presence of micelles.

In MLC, retention and selectivity are governed by the three competing equilibria: partitioning from bulk solvent to micelles and to the stationary phase, or direct transfer from micelles to the stationary phase. Equation 7.1 can be rewritten in logarithmic form as:

$$\log k = \log (\Phi P_{\text{ws}}) - \log (1 + K_{\text{AM}} [M]) \quad (7.9)$$

If the relationships between both $\log (\Phi P_{\text{ws}})$ and $\log (1 + K_{\text{AM}} [M])$ with ϕ are linear, the following can be expressed:

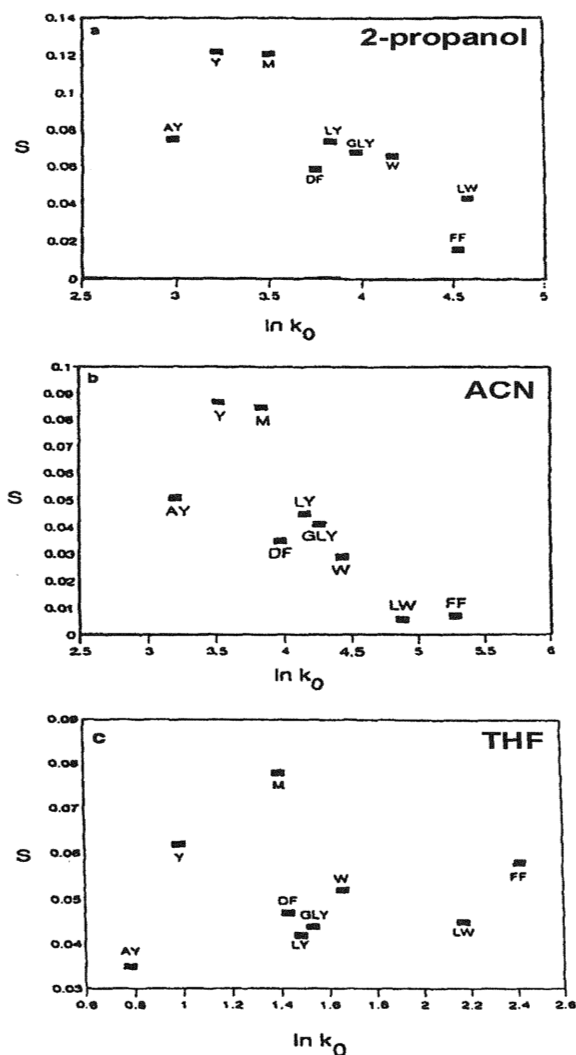


Figure 7.14 Relationship between S_{hyb} and $\log k'_0$ in eq. 7.8 for diverse amino acids and peptides, eluted with SDS mobile phases containing: (a) 2-propanol, (b) acetonitrile, and (c) tetrahydrofuran. Compounds: alanyl-tyrosine (AY), tyrosine (Y), methionine (M), leucyl-tyrosine (LY), aspartyl-phenylalanine (DF), glycyl-leucyl-tyrosine (GLY), tryptophan (W), leucyl-tryptophan (LW), phenylalanyl-phenylalanine (FF). Reprinted from Ref. 10 with permission of Elsevier.

$$\log (\Phi P_{ws}) = \log (\Phi P_{wso}) - S\varphi \quad (7.10)$$

$$\log (1 + K_{AM} [M]) = \log (1 + K_{AMO} [M]) - S_m\varphi \quad (7.11)$$

where ΦP_{wso} and K_{AMO} are the partition coefficients for pure aqueous micellar eluents [15]. The parameters S_s and S_m represent the sensitivity of variations in solute partitioning from bulk solvent into the stationary phase and into the micelles, respectively, with changes in φ . From equations 7.1 and 7.9-7.11, it is derived that S_{hyb} is dependent upon S_s and S_m , according to:

$$S_{hyb} = S_s - S_m \quad (7.12)$$

The negative sign in equation 7.12 clearly reflects the competing nature of the two partitioning equilibria (into stationary phase and micelles). In the absence of micelles, $S_m = 0$ and $S_{hyb} = S_s$, which represents the solvent strength parameter in conventional aqueous-organic RPLC. This equation also shows that the elution strength (i.e., S values) in hybrid micellar systems will be generally smaller than in aqueous-organic eluents.

VI.2. *Ranking of Elution Strength of Organic Modifiers*

A widely accepted technique for characterizing solvents, in liquid chromatography, is the Snyder's selectivity triangle [16]. This technique classifies organic solvents on the basis of their relative ability to engage in proton accepting, proton donating and strong dipolar interactions. When the resulting values are plotted on three axes in the form of a triangle, solvents having similar functionalities tend to fall within the same area of the triangular plot (e.g., 2-propanol and 1-butanol belong to group II of Snyder's triangle; tetrahydrofuran to group III, and acetonitrile to group VIb). In principle, the solvents grouped in the same area of the triangle should have similar chromatographic selectivity, while solvents from other groups should exhibit different selectivity, for a given separation. This theory has been

widely accepted and has often formed the rationale for solvent selection for optimizing a given RPLC separation.

The presence of micelles in the mobile phases of RPLC has great influence on the chromatographic selectivity of organic solvents. As a result, the classification established by Snyder seems to be no longer valid in MLC with hybrid mobile phases. Thus, according to Snyder's solvent classification, the chromatographic selectivity for 2-propanol and 1-butanol in aqueous-organic mobile phases should be the same at equal elution strengths, because they belong to the same selectivity group. The *S* values of some amino acids and peptides, for 2-propanol-water and 1-butanol-water, are given in Table 7.2 [10]. The compounds are ranked according to the *S* values in 2-propanol-water mobile phases. As expected, the *S* values in 1-butanol-water are larger than those for 2-propanol-water, and the ranks of *S* values of different solutes for both alcohols are the same. In other words, the selectivity of solutes in 2-propanol-water and 1-butanol-water mobile phases will be similar at equal elution strengths.

Table 7.2 Solvent Strength Parameters for 1-Propanol-Water and 1-Butanol-Water Mobile Phases, Without Surfactant and With 0.02 M SDS [10]

Compound	2-Propanol-Water	1-Butanol-Water	2-Propanol-0.02 M SDS	1-Butanol-0.02 M SDS
Aspartyl-phenylalanine	14.1	35.5	8.77	25.7
Tryptophan	16.3	36.3	11.8	34.0
Leucyl-tyrosine	17.5	37.1	8.77	22.1
Glycyl-leucyl-tyrosine	18.4	44.6	7.49	21.1
Leucyl-tryptophan	18.7	50.2	7.07	23.3
Phenylalanyl-phenylalanine	20.4	53.2	7.32	28.3

The S_{hyb} values for the same test solutes in the hybrid systems of 2-propanol-SDS and 1-butanol-SDS, at a micelle concentration of 0.02 M, are also shown in Table 7.2. Since, in MLC, organic modifiers associate with micelles and, on the other hand, compete with micelles to interact with the solutes, the rank of S_{hyb} values of some solutes for 2-propanol is different from that for 1-butanol. For example, S_{hyb} for phenylalanyl-phenylalanine with 2-propanol is the second lowest and with 1-butanol is the second highest, or S_{hyb} for leucyl-tyrosine for 2-propanol is the second highest, while for 1-butanol is the second lowest. Also, in a study performed with 11 aromatic compounds, in methanol-water and hybrid CTAB-methanol eluents [14], anthracene showed $S = 12.6$ (the largest), benzyl alcohol was ranked ninth with $S = 5.05$, and phenol had the smallest value of 3.13. As a result, the retention of anthracene in conventional aqueous-organic RPLC was more sensitive to variations in the concentration of organic solvent than benzyl alcohol or phenol. In the presence of CTAB micelles, however, the corresponding S_{hyb} values and the ranking were quite different and, in fact, opposite to that of conventional aqueous-organic eluents. Thus, $S_{\text{hyb}} = 0.47$ for anthracene (ranked tenth, one of the smallest), 1.53 for benzyl alcohol (the largest), and 1.31 for phenol (ranked fourth).

A comparison of the values of S and S_{hyb} shows that the elution strength decreased due to the inclusion of micelles in the aqueous-organic media (i.e., $S_{\text{hyb}} < S$). The smaller variations in S_{hyb} mean that solute size is less important compared to the solvating effect of the alcohol modifier. The magnitude of the reduction in elution strength depends upon the degree of interactions of solutes and organic solvents with micelles. Micelles control the solvation ability of organic solvents and, as a result, their chromatographic selectivity. Consequently, one can expect that the ranks and the magnitudes of S_{hyb} for the alcohols also change with micelle concentration.

In Table 7.3, the S_{hyb} values for some PAHs of environmental concern, with a wide range of hydrophobicity, are listed for methanol, 2-propanol and 1-butanol as modifiers, with SDS and CTAB micelles. As shown, the S_{hyb} values can be ranked as:

$$S_{\text{butanol}} > S_{\text{propanol}} > S_{\text{methanol}}$$

Table 7.3 Absolute Values of the Slopes of $\ln k$ vs. Percentage of Organic Modifier, Calculated by Linear Regression, for Several PAHs Eluted with Hybrid Eluents of 0.15 M SDS-Alcohol and 0.02 M CTAB-Alcohol [17].

Compound	SDS 0.15 M			CTAB 0.02 M		
	Methanol	2-Propanol	1-Butanol	Methanol	2-Propanol	1-Butanol
Dibenz[<i>ac</i>]anthracene	2.7	2.7	8.3	1.9	2.5	8.8
Benzo[<i>ghi</i>]perylene	2.7	2.9	7.9	1.3	2.2	8.0
Fluoranthene	2.4	3.1	7.9	1.3	1.9	9.1
Chrysene	2.3	3.1	8.1	1.7	2.1	7.2
Benzo[<i>a</i>]anthracene	2.3	2.9	8.2	1.4	1.9	7.4
Benzo[<i>e</i>]pyrene	2.1	2.8	7.6	1.4	2.6	6.4
Perylene	2.1	2.9	8.1	1.6	2.7	6.7
Benzo[<i>a</i>]pyrene	2.0	3.0	8.7	1.5	2.2	6.7
Pyrene	2.0	3.2	8.0	1.4	2.5	7.9
9-Methylanthracene	1.9	3.0	7.3	1.4	2.6	8.7
Benzo[<i>b</i>]fluoranthene	1.9	2.9	7.9	2.0	2.4	6.9
Phenanthrene	1.8	3.0	8.3	1.2	3.2	9.2
Anthracene	1.8	3.2	8.0	1.2	3.1	9.6
Naphthalene	1.8	3.3	7.3	1.4	4.1	6.9
Acenaphthylene	1.7	3.2	7.5	1.5	4.2	6.3
Fluorene	1.6	2.9	8.0	1.4	3.2	10.2

which is similar to conventional aqueous-organic systems, as 1-butanol is the strongest solvent and methanol the weakest. The larger S_{hyb} for 1-butanol (3-5 times) corroborates that this solvent interacts more strongly with micelles and can compete better for the interaction with solutes. Because each alcohol modifier interacts differently with micelles, the selectivity changes from one another. Finally, the different impact of micelles of SDS and CTAB on the ranking of solutes, and the importance of solute size and hydrophobicity should be noted.

VI.3. Simultaneous Enhancement of Elution Strength and Selectivity with Hybrid Micellar Eluents

As commented above, in conventional RPLC, a systematic decrease in selectivity occurs usually as a result of an increase in volume fraction of organic modifier (i.e., elution strength). In contrast, in the presence of micelles, the selectivity may increase, decrease or remain unchanged with elution strength. The elution strength may thus be enhanced without sacrificing the selectivity. It should be reminded that improving the resolution and reducing the separation duration are the two important goals in many optimization strategies. Simultaneous selectivity enhancement with elution strength can lead to a better separation in a shorter time period.

The simultaneous enhancement in elution strength and selectivity, often observed in MLC, can be attributed to the existence of the three competing equilibria. Examples are given by the separation of a mixture of amino acids and small peptides, and a mixture of substituted benzenes, eluted with hybrid eluents of SDS and 2-propanol (Figs. 7.15 and 7.16) [15]. Figure 7.15 shows the influence of the concentration of 2-propanol on selectivity, for several pairs of compounds at a constant micelle concentration. Selectivity variations occur systematically and monotonically for different peaks, as a result of an increase in 2-propanol phase content. Figure 7.16 illustrates the changes in selectivity for the same pairs of compounds due to the variations in micelle concentration, at a constant 2-propanol composition. Interestingly, micelle concentration had an opposite effect on selectivity as compared to 2-propanol. For those pairs of peaks whose selectivity were reduced with increasing 2-propanol content, an enhancement in selectivity was observed as a result of increasing micelle

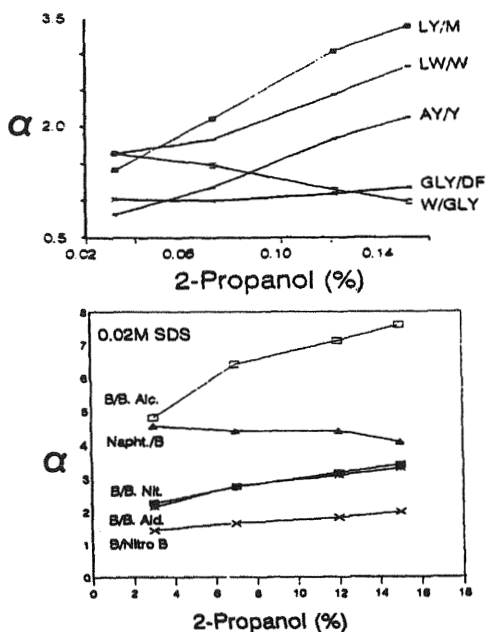


Figure 7.15 Variation in selectivity with volume fraction of 2-propanol at 0.02 M SDS, for amino acids and peptides (top), and substituted benzenes (bottom). Compounds: benzene (B), benzyl alcohol (B Alc.), naphthalene (Napht.), benzonitrile (B. Nit.), benzaldehyde (B. Ald.), nitrobenzene (Nitro B). See other symbols in Fig. 7.14. Reprinted from Ref. 15 with permission of the American Chemical Society.

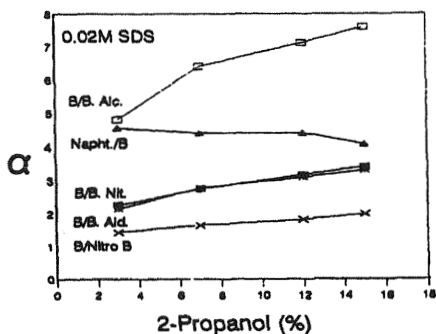
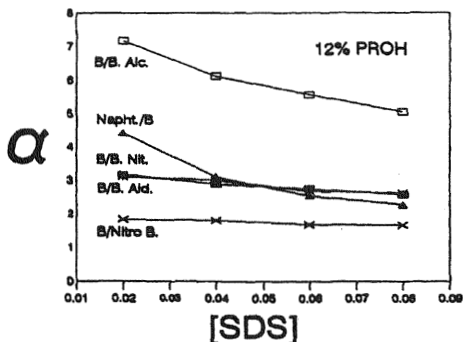
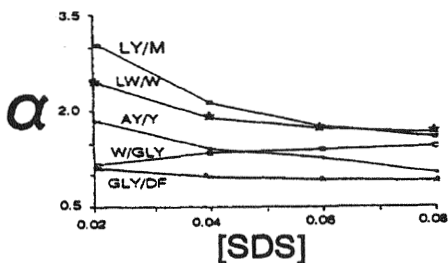


Figure 7.16 Variation in selectivity with micelle concentration at 12% 2-propanol, for amino acids and peptides (top) and substituted benzenes (bottom). See symbols in Figs. 7.14 and 7.15. Reprinted from Ref. 15 with permission of the American Chemical Society.



concentration and vice versa. These observations suggest that although the elution strength increases with the concentration of both micelle and organic solvent, the effect of both on selectivity could be quite different, even opposite. Micelles and 2-propanol compete to interact with solutes, and as a result, they influence the role of one another in controlling the retention and selectivity.

An expression describing the selectivity in MLC can be derived from eq. 7.1:

$$\alpha = \frac{\alpha_{WS}}{\alpha_{AM}} \quad (7.13)$$

where α_{AM} is a function of the binding selectivity to micelles:

$$\alpha_{AM} = \frac{1 + K_{AM,2} [M]}{1 + K_{AM,1} [M]} \quad (7.14)$$

and α_{WS} is the stationary-phase partitioning selectivity:

$$\alpha_{WS} = \frac{P_{WS,2}}{P_{WS,1}} \quad (7.15)$$

and have opposite effects on chromatographic selectivity.

The values of K_{AM} and P_{WS} usually decrease with an increase in volume fraction of modifier. The degree of decrease of these parameters, however, is not equal for different solutes. This can lead to changes in selectivity. To study this behavior, the partition coefficients of a group of compounds (i.e., amino acids, peptides and benzene derivatives) were measured at different concentrations of 2-propanol. For those pairs of compounds for which the selectivity increased as a result of increasing 2-propanol, the partition coefficients of the less retained compound in the pair decreased to a higher degree, as compared to the more retained compound. For other pairs of solutes for which the selectivity decreased with an increase in 2-propanol, the reduction in the coefficients of the compound showing the

higher retention in the pair was larger than for the compound with lower retention. Figure 7.17 illustrates variations in K_{AM} and P_{WS} for three solutes, with an increase in percentage of 2-propanol. As shown, the rank of the slopes for these solutes is tryptophan (W) > alanyl-tyrosine (AY) > leucyl-triptophan (LW). Leucyl-triptophan /tryptophan (LW/W) and leucyl-triptophan/alanyl-tyrosine (LW/AY) belong to the first group of solute pairs (for which the selectivity increases as a result of an increase in organic solvent concentration), and W/AY belongs to the second group of solute pairs (for which the selectivity decreases as a result of an increase in organic solvent concentration).

The variations in α_{WS} and α_{AM} for the three pairs of solutes, with increasing 2-propanol, are shown in Figure 7.18 (top and middle). The selectivity is controlled by the competition between these two parameters (eq. 7.13). Figure 7.18 (bottom) illustrates the variation in chromatographic selectivity with volume fraction of organic modifier. It is shown in the figure that there is a systematic change in selectivity with organic solvent concentration, which may increase or decrease.

Another important factor is the effect of micelle concentration on α_{AM} , and therefore, on chromatographic selectivity. For the first group of pairs of compounds, like LW/W, both α_{WS} and α_{AM} increase with 2-propanol, but the rate of increase of the former is greater (Fig. 7.18). However, when micelle concentration increases at fixed 2-propanol, the rate of increase in α_{AM} becomes larger than the rate of increase in α_{WS} , and the selectivity decreases. For the second group of solutes, like W/AY, both α_{WS} and α_{AM} decrease with an increase in 2-propanol, but the degree of decrease in α_{WS} is more than that of α_{AM} . As a result of an increase in micelle concentration at fixed percent 2-propanol, the degree of decrease in α_{AM} becomes larger than that of α_{WS} , and the selectivity increases.

In aqueous-organic systems, it is common practice to first adjust the elution strength, and then optimize the selectivity at a constant elution strength. In hybrid systems, where an increase in elution strength can often coincide with an enhancement in selectivity, a separate optimization of elution strength and selectivity is inefficient. This is the main subject treated in Chapter 8, where it is shown how the simultaneous optimization of parameters influencing elution strength and selectivity can be performed.

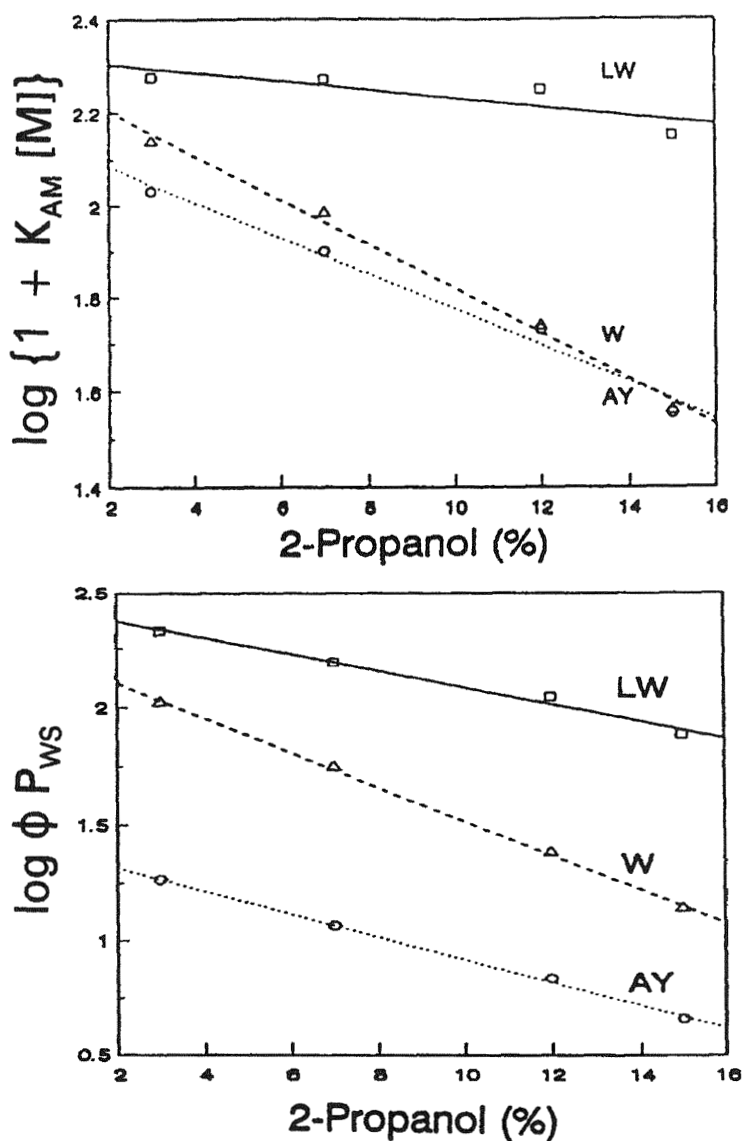


Figure 7.17 Linear regression of $\log \{1 + K_{AM}[M]\}$ and $\log \{\phi P_{ws}\}$ vs. volume fraction of 2-propanol for: leucyl-triophan (LW), tryptophan (W), and alanyl-tyrosine (AY). Reprinted from Ref. 15 with permission of the American Chemical Society.

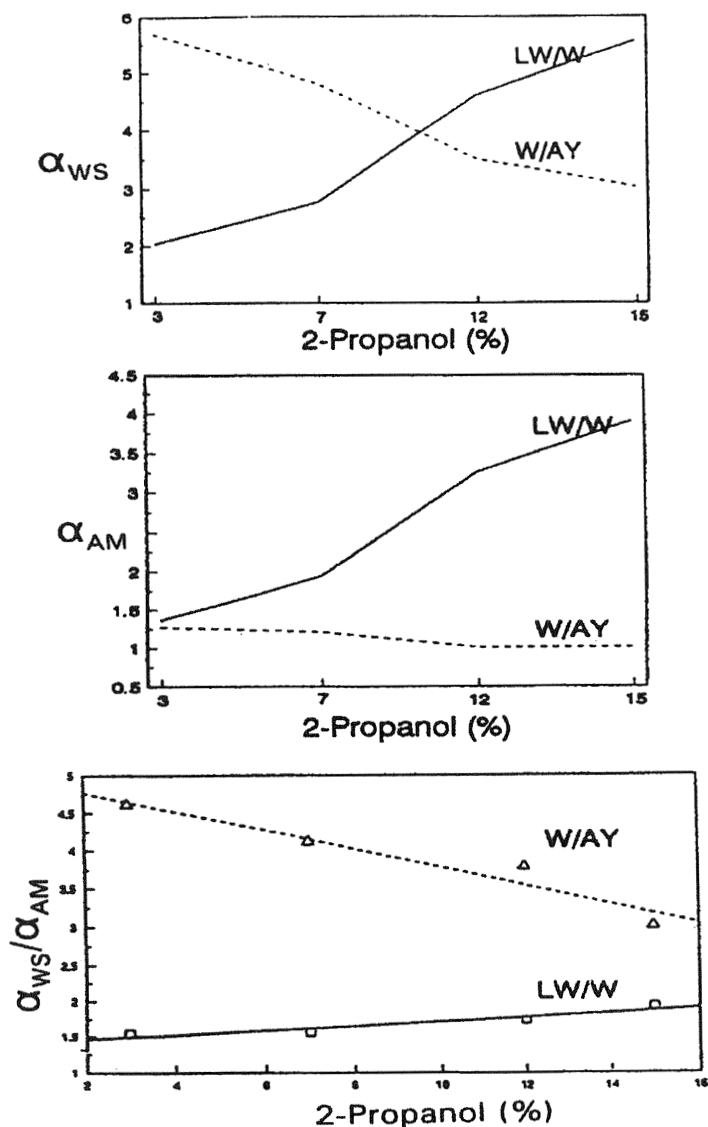


Figure 7.18 Variation in α_{WS} , α_{AM} , and linear regression of α_{WS}/α_{AM} vs. volume fraction of 2-propanol for: leucyl-triophan/tryptophan (LW/W) and tryptophan/alanyl-tyrosine (W/AY). The concentration of SDS in the mobile phases was 0.02 M. Reprinted from Ref. 15 with permission of the American Chemical Society.

VII. INFLUENCE OF TEMPERATURE ON SELECTIVITY

The effect on selectivity of increasing the temperature of the chromatographic column is usually ignored, perhaps because the improvements in selectivity are not very substantial. However, with highly hydrophobic solutes, the influence of temperature may be important since the rate of transfer of solute between bulk aqueous phase and stationary phase, or between micellar pseudo-phase and stationary phase, will probably be slow. An increased temperature will favor the transfer processes.

Figure 7.19 shows the chromatogram of a test mixture of nine PAHs. At 30°C, the mixture was unresolved and all peaks showed bad symmetry and efficiency, especially for the most hydrophobic solutes (peaks 4-15). At 40°C, the separation was still poor. At 50°C, the improvement was evident, the efficiency of the separation of peaks 8-15 increased considerably. The best situation occurred at 60°C, where the mixture was totally resolved and the analysis time diminished in comparison with the initial situation at 30°C. The enhanced silica dissolution is a problem that should not be neglected when working at elevated temperature with micellar phases.

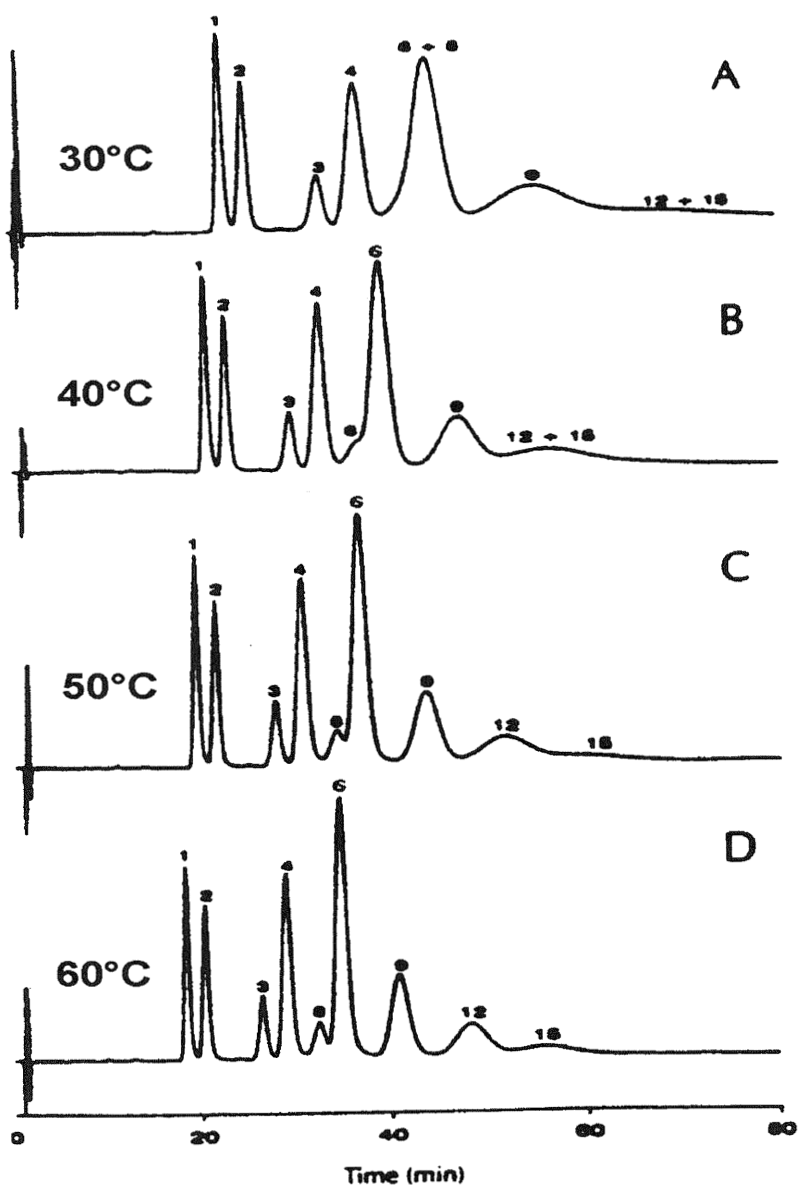


Figure 7.19 Chromatograms of a mixture of PAHs, using 0.15 M SDS as mobile phase. Temperature: (A) 30°C, (B) 40°C, (C) 50°C and (D) 60°C. The compounds are the same as in Fig. 7.10. Reprinted from Ref. 17 with permission of Elsevier.

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Modeling of the Retention Behavior

I. INTRODUCTION

1.1. Use of an Optimization Strategy in Liquid Chromatography

Chapter 5 showed how the presence of micelles in a Reversed-Phase Liquid Chromatographic (RPLC) system can provide a great variety of interactions (Fig. 5.1). In the micellar mobile phases, the solutes can remain outside the micelle associated with the polar head of the surfactant, can form a part of the outer palisade layer, or can penetrate into the micelle core. Also, the monomers of ionic surfactants can be adsorbed on alkyl-bonded stationary phases, mainly through hydrophobic interaction between the tail of the surfactant and the alkyl chains of the stationary phase. In this case, the charged head of the surfactant will remain in contact with the polar solution. Solutes can experience hydrophobic interactions with either nonpolar tails of the adsorbed surfactant and/or nonpolar bonded moieties of the stationary phase, and polar interactions with the ionic head of the adsorbed surfactant and free silanol groups on the stationary phase. Nonpolar solutes will only be affected by hydrophobic interactions with both micelles and stationary phase, but charged solutes will give rise to two distinct additional situations, according to the sign of their charge, which can be the same or opposite to the sign of the head of the surfactant and can, therefore, be repelled or attracted by the surfactant.

Most reported procedures for the determination of compounds in Micellar Liquid Chromatography (MLC) make use of micellar mobile phases containing an organic modifier, usually a short-chain alcohol or acetonitrile. These modifiers increase the elution strength, which is particularly important for the most hydrophobic solutes, and often improve the shape of the chromatographic peaks. The most hydrophilic alcohols do not penetrate the micelles, but butanol and pentanol can be inserted into the

micelle with their hydroxyl group orientated towards the Stern layer, and their hydrocarbon chain remaining inside the nonpolar micelle core. The their hydrocarbon chain remaining inside the nonpolar micelle core. The incorporation of the alcohol in the micelle can result in additional interactions with the solutes. On the other hand, the modifiers solvate the bonded stationary phase and reduce the amount of surfactant adsorbed, the effect being larger with increasing concentration and hydrophobicity of the alcohol. The rigidity of the surfactant-alkyl-bonded ligand structure may also be affected.

A decrease in retention times is usually observed when either the micelle concentration or the concentration of organic solvent is increased. However, different components in a mixture respond in different ways to changes in concentration of surfactant and/or organic modifier, resulting in changes in resolution. Selection of pH in the mobile phase is also often extremely important for the resolution of complex mixtures, owing to the side acid-base reactions of many solutes. Other variables to be considered are temperature and ionic strength.

The chromatographer is concerned with the achievement of the optimum mobile phase that permits the separation of the compounds in a mixture, in the minimum time. This task may be really difficult when two or more variables are involved in the optimization process. The optimization strategy utilized may be sequential or interpretive. In a sequential strategy, the retention of the solutes is not known *a priori*, and each set of mobile phases is designed by taking into account the retention observed with previous eluents. In contrast, in an interpretive strategy, the experiments are designed before the optimization process and used to fit a model that will permit the prediction of the retention of each solute. This strategy may be much more efficient and reliable. A sequential strategy will be inadequate when several local (or secondary) maxima exist (as occurs in chromatography), and may not give the best maximum, that is, the optimum.

The necessity for an adequate experimental design becomes especially important when dealing with forms of liquid chromatography suitable for the simultaneous analysis of ionic and nonionic compounds, such as MLC, where several variables should be controlled (*i.e.*, type and concentration of surfactant and organic modifier, pH, temperature and ionic strength). The method development strategy must provide the chromatographer with an answer to which variables (factors) should be used, and how

to set up initial experiments to search the appropriate variable space in an efficient way.

The separation process in a micellar chromatographic system requires a structured approach in the development of practical applications. Ideally, the resolution of complex mixtures should be made and optimized in a short time, with minimal consumption of reagents.

The reader should not be repulsed by the somewhat complex equations developed in this chapter. They are incorporated in the *MICHRUM* software supplied with the book. Appendix I describes the computer assisted way to model the retention behavior of a given mixture in MLC. With a few guided experiments, the software will be able to model the retention of an actual mixture components with surprising accuracy.

1.2. Predictable Retention Behavior

As commented above, in MLC the chromatographer is supplied with several tools to refine a separation and optimize the resolution for a given multi-component mixture. However, this advantage can only be exploited to the fullest when all variables are taken into account simultaneously. By only using the proton, surfactant, or organic modifier concentration, or by varying one after optimizing the other, the best separation can easily be missed. The use of an interpretive optimization strategy, which needs specific information on the retention of the individual components in a mixture, may require a relatively low number of experiments to derive an acceptable separation.

The accurate prediction of the retention of each compound in a given mixture is more important in MLC than in conventional RPLC, due to the negative effect of the broadening and distortion of the chromatographic peaks for some solutes and eluent compositions, with micellar mobile phases. Fortunately, as shown in this chapter, solute retention in MLC can be accurately predicted. A regular change in the retention behavior is observed with the variation of the concentrations of surfactant and organic modifier, and pH. The simultaneous optimization of the resolution and analysis time is thus possible, on the basis of a limited number of experiments, even though these experiments were relatively far apart in the variable space. As seen below, modeling of the retention behavior in MLC has also allowed a

better understanding of the processes occurring inside the chromatographic system.

I.3. Selection of the Variable Space

The boundaries of the variables included in the modeling process should be set by the operator, on the basis of previous experience. The extreme values are imposed by the practical limitations of the chromatographic system: the lower surfactant concentration must be well above the critical micellar concentration (cmc), and strong enough to cause elution of all components. The upper surfactant concentration is determined by a combination of the solubility of the surfactant, the acceptable viscosity of the resulting mobile phase (i.e., maximum pressure drop over the column), and the degradation of the efficiency at higher concentrations. The concentration of organic modifier must consider the retention times, and is limited to a maximum to ensure the integrity of micelles. Finally, the pH range for a silica reversed-phase support, between 2.5 and 7.5, should be considered.

When the concentration of organic modifier becomes too high, the characteristics of the micellar pseudo-phase change: a microemulsion can be created or the micelles can completely disappear. The maximum concentrations of modifier reported for modeling purposes, with sodium dodecyl sulfate (SDS) as surfactant, have been 15% propanol, 6% butanol, 3% pentanol, and 20% acetonitrile (v/v concentrations).

II. RETENTION BEHAVIOR IN PURE MICELLAR ELUENTS

II.1. Micelle Concentration as Unique Experimental Variable

The three-phase theory [1], which postulates the following equilibria between a solute, A, the micelle, M, and the stationary phase, S:



predicts the retention factors, k , in micellar media at a given pH, according

$$\frac{1}{k} = \frac{1}{K_{AS}} + \frac{K_{AM}}{K_{AS}} [M] \quad (8.3)$$

to a very simple equation:

which relates the retention with the concentration of monomers of surfactant in the form of micelles, $[M]$. In this equation, K_{AM} is the binding constant between solute and micelle, and K_{AS} the partition coefficient between stationary phase and water multiplied by the phase ratio (see Chapter 5). This equation can be rewritten as:

$$\frac{1}{k} = c_0 + c_1 [M] \quad (8.4)$$

It has been extensively commented in Chapter 5 that eq. 8.3 has been verified, experimentally, for a large number of solutes (ionic, and polar and nonpolar neutral), different types of surfactants (anionic, cationic and nonionic), and diverse column materials (mainly C8, C18 and cyano). It is also valid for mobile phases containing an organic modifier. Deviations from the model are, however, observed for very highly and poorly retained solutes.

II.2. Gradient Elution

The separation of mixtures of compounds showing a wide range of polarities can be advantageously made by using a gradient of ionic surfactant. In MLC, gradient elution is favored because at moderate concentrations of ionic surfactant, the composition of the stationary phase remains constant during the gradient. Therefore, the only reequilibration process necessary, before the next gradient run, is flushing the chromatographic system with the initial mobile phase. Prediction of the retention in gradient conditions in MLC has been made based on the gradient elution theory developed by

Snyder [2], assuming the linear model given by eq. 8.3 and a linear change in micelle concentration [3]:

$$[M] = a + bV \quad (8.5)$$

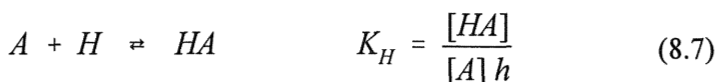
V being the volume of the mobile phase delivered. The equation finally derived was:

$$t_g = \frac{t_o K_{AS}}{K_{AM} b V_o} \left(-\frac{1}{k_1} + \sqrt{\left(\frac{1}{k_1} \right)^2 + 2 \frac{K_{AM} b V_o}{K_{AS}} \left(1 - \frac{t_D}{t_o k_1} \right)} \right) + t_o + t_D \quad (8.6)$$

where t_g is the gradient retention time, t_o the dead time, V_o/t_o the volume flow rate, k_1 the retention factor of the solute at the initial mobile phase, and t_D the delay time (time before the gradient actually reaches the top of the column). This model has shown excellent results for a variety of solutes and SDS gradients in the 0.10-0.50 M range.

II.3. Simultaneous Effect of pH and Micelle Concentration

Method development in MLC can be largely potentiated by extending the modeling of the retention along the pH scale (Fig. 8.1). Arunyanart and Cline-Love [4], and Rodgers et al. [5,6], studied the effect of pH on the MLC retention of weak acids and bases. These authors combined the equations that describe the acid-base equilibrium for a monoprotic system in water:



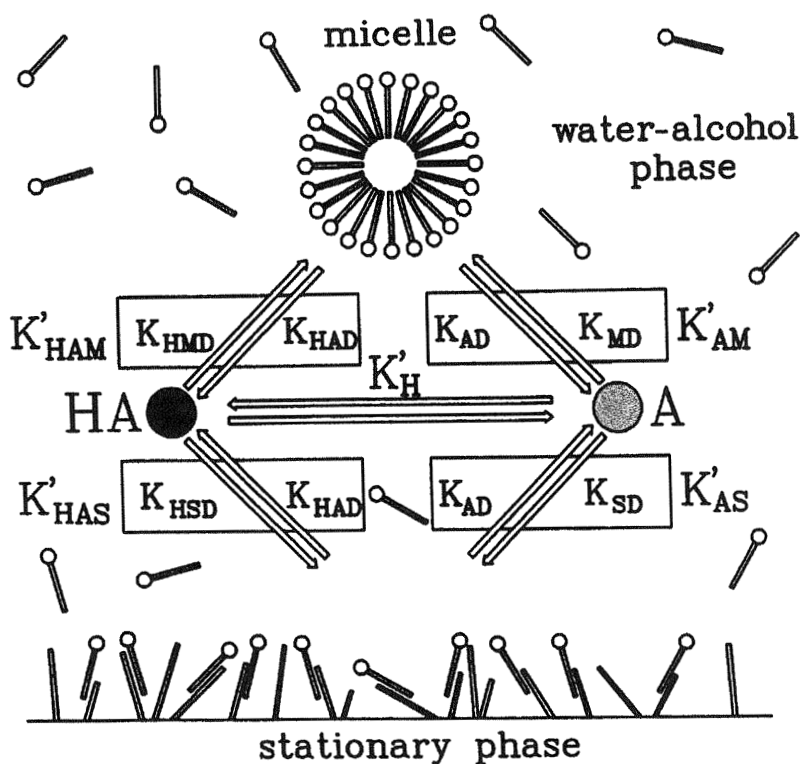


Figure 8.1 Solute-micelle and solute-stationary phase interactions in micellar mobile phases of surfactant and organic modifier, at varying pH.

and the retention factor in a micellar chromatographic system:

$$k = \phi \frac{[AS] + [HAS]}{[A] + [HA] + [AM] + [HAM]} \quad (8.8)$$

where h is proton concentration, K_H is the protonation constant, $[AS]$, $[AM]$ and $[A]$ refer to the basic species, and $[HAS]$, $[HAM]$ and $[HA]$ to the acidic

species. By substituting eq. 8.7 and the equilibrium constants relating all these species (see equilibria 8.1 and 8.2) in eq. 8.8, the following is given:

$$k = \frac{K_{AS} + K_{HAS} K_H h}{1 + K_H h + K_{AM} [M] + K_{HAM} K_H h [M]} \quad (8.9)$$

Rewriting this expression, an equation similar to the reciprocal of eq. 8.3 is obtained:

$$k = \frac{\frac{K_{AS} + K_{HAS} K_H h}{1 + K_H h}}{1 + \frac{K_{AM} + K_{HAM} K_H h}{1 + K_H h} [M]} = \frac{K_{AS}^H}{1 + K_{AM}^H [M]} \quad (8.10)$$

where K_{AS}^H and K_{AM}^H are apparent constants with respect to proton concentration. Equation 8.9 may also be rewritten as follows:

$$k = \frac{\frac{K_{AS}}{1 + K_{AM} [M]} + \frac{K_{HAS}}{1 + K_{HAM} [M]} \times \frac{1 + K_{HAM} [M]}{1 + K_{AM} [M]} K_H h}{1 + \frac{1 + K_{HAM} [M]}{1 + K_{AM} [M]} K_H h} \quad (8.11)$$

or

$$k = \frac{k_A + k_{HA} K_H^M h}{1 + K_H^M h} \quad (8.12)$$

where k_A and k_{HA} are the retention factors of the basic and acidic species, respectively. Equations 8.11 and 8.12 indicate that the retention varies with

pH, following a sigmoidal behavior between the retention of the acidic species and the basic species. The effect of the concentration of surfactant on the apparent protonation constant is also shown in eq. 8.11.

This equation may easily be extended to solutes exhibiting several protonation equilibria in the pH working range of reversed-phase columns. For a diprotic system, the retention as a function of micelle concentration and pH will be given by:

$$k = \frac{\frac{K_{AS}}{1+K_{AM}[M]} + \frac{K_{HAS}}{1+K_{HAM}[M]} \frac{1+K_{HAM}[M]}{1+K_{AM}[M]} K_H^{-1} h + \frac{K_{H2AS}}{1+K_{H2AM}[M]} \frac{1+K_{H2AM}[M]}{1+K_{AM}[M]} K_I}{1 + \frac{1+K_{HAM}[M]}{1+K_{AM}[M]} K_H^{-1} h + \frac{1+K_{H2AM}[M]}{1+K_{AM}[M]} K_H^{-1} K_H^{-2} h^2} \quad (8.13)$$

where K_H^{-1} and K_H^{-2} are the consecutive protonation constants, and K_{H2AS} and K_{H2AM} the partition constants of the diprotonated species.

Figure 8.2 shows simulated retention of zwitterionic compounds in MLC, based upon eq. 8.13, with nonionic and anionic micelles [6]. The retention behavior with nonionic micelles (Fig. 8.2, top) is similar to conventional RPLC, with the retention passing through a minimum. For anionic micelles (Fig. 8.2, bottom), electrostatic repulsion between the solute and surfactant will result in low retention at elevated pH. For both nonionic and anionic surfactants, the retention decreases with increasing micelle concentration. It is important to note that a large degree of error in predicting the retention is to be expected as a result of small errors in pH measurements, in the region where the solute is being protonated.

For monoprotic systems, the apparent protonation constant can be determined by measuring the retention factor at several pH values and fitting the experimental data to eq. 8.12, via nonlinear regression. A similar equation can be used for diprotic systems. Figure 8.2 shows micellar-induced shifts of the apparent protonation constants, with increasing anionic micelles. The logarithm of the first protonation constant ($\log K_H = \text{p}K_a$) for tryptophan, phenylalanine and lysine in water is approximately 2.4. For the micellar mobile phase, $\log K_H^M$ are between 3.60 and 4.56. Therefore, a

greater range of protonation constants exists in the micellar mobile phase. This results in larger changes in selectivity with pH in MLC due to the differential shift in protonation constants between the solutes, which increases the importance of pH in the optimization process.

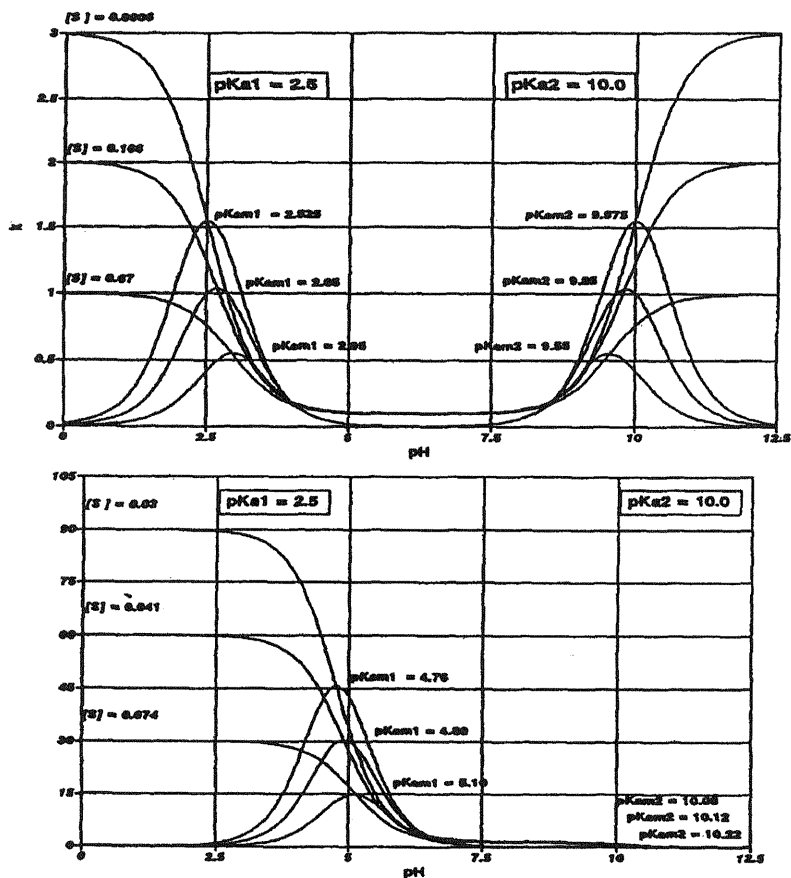


Figure 8.2 Predicted retention of a zwitterion (pK_a and pK_{am} are acid-base dissociation constants in water and micellar solution, respectively, and $[S]$ is surfactant concentration), as a function of pH and micelle concentration with nonionic micelles (top): solute-micelle association constants for cation, zwitterion and anion are 3, 0.1 and 3, respectively, $cmc = 2.4 \times 10^{-4}$ M; and with anionic micelles (bottom): solute-micelle association constants are 10,000, 10 and 0.1, respectively, $cmc = 8.3 \times 10^{-3}$ M. The derivatives of the sigmoidal curves at each micelle concentration are also shown to give the apparent dissociation constants. Reprinted from Ref. 6 with permission of the American Chemical Society.

III. RETENTION BEHAVIOR IN HYBRID MICELLAR ELUENTS AT CONSTANT pH

III.1. *A Model for Conventional RPLC*

As a result of the increasing use of modifiers in MLC, the need for an adequate description of the retention in hybrid micellar mobile phases appeared. Khaledi et al. [7, 8] were the first which intended to model the retention of solutes in these systems. They assumed that the linear relationship between $\log k$ and the volume fraction of organic modifier, ϕ , followed in conventional RPLC over a small range of values of ϕ , was also valid in hybrid MLC at constant micelle concentration:

$$\log k = \log k_0 - S\phi \quad (8.14)$$

According to these authors, $\log k_0$ in eq. 8.14 was the logarithm of the retention factor at a given micelle concentration in the absence of modifier. However, linear relationships are only actually obtained with methanol as modifier, probably owing to its weak elution strength. Figure 8.3 shows $\log k$ vs. ϕ plots for several aminochromes [9]. With the data plotted, excluded the point for $\phi = 0$, the value of k_0 was calculated from the intercept of the fitted straight-line according to eq. 8.14. It was found that the difference between the experimental and calculated k_0 was larger for an increasing alkyl chain-length of alcohols. Curiously, a linear relationship existed between this difference and the number of carbon atoms in the alcohol.

III.2. *Iterative Regression Strategy*

In order to model the retention in a hybrid micellar mobile system, Strasters et al. [8] proposed a procedure that used the retention data of only five mobile phases: four measurements at the corners of the selected two-dimensional variable space, defined by the concentrations of surfactant and modifier, and the fifth in the center (design VI in Fig. 8.4). In this method, the rectangular variable space is divided into four triangular subspaces defined by three of the five measurements: two neighbor corner

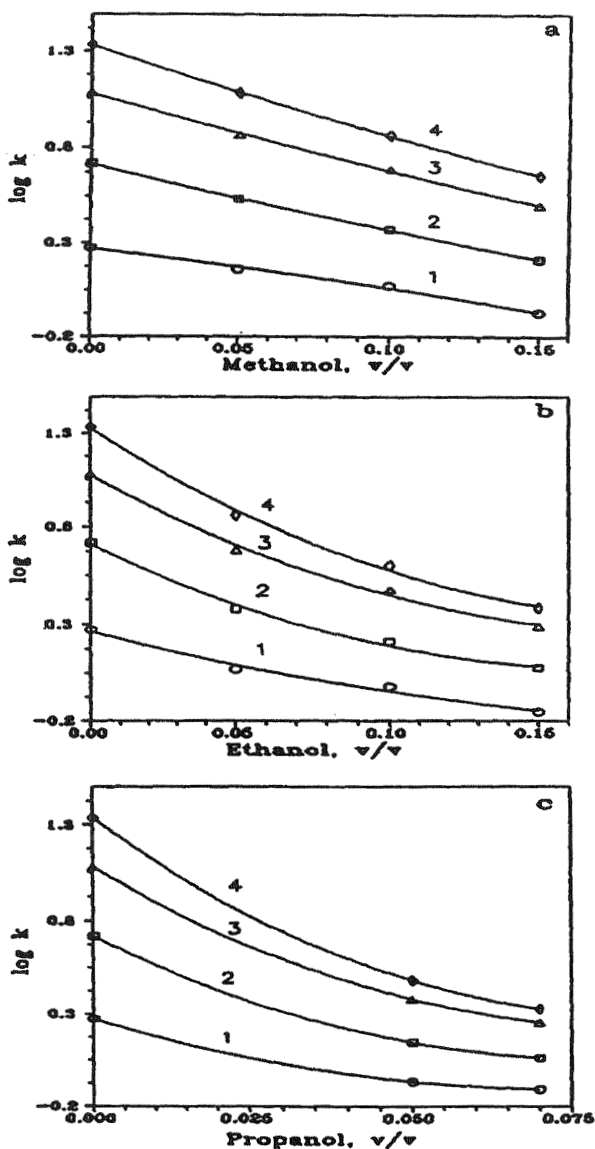


Figure 8.3 $\log k$ vs. ϕ plots for aminochromes: (1) noradrenochrome, (2) adrenochrome, (3) dopaminechrome, and (4) isopropylnoradrenochrome. The mobile phase contained 0.05 M SDS. Reprinted from Ref. 9 with permission of Elsevier.

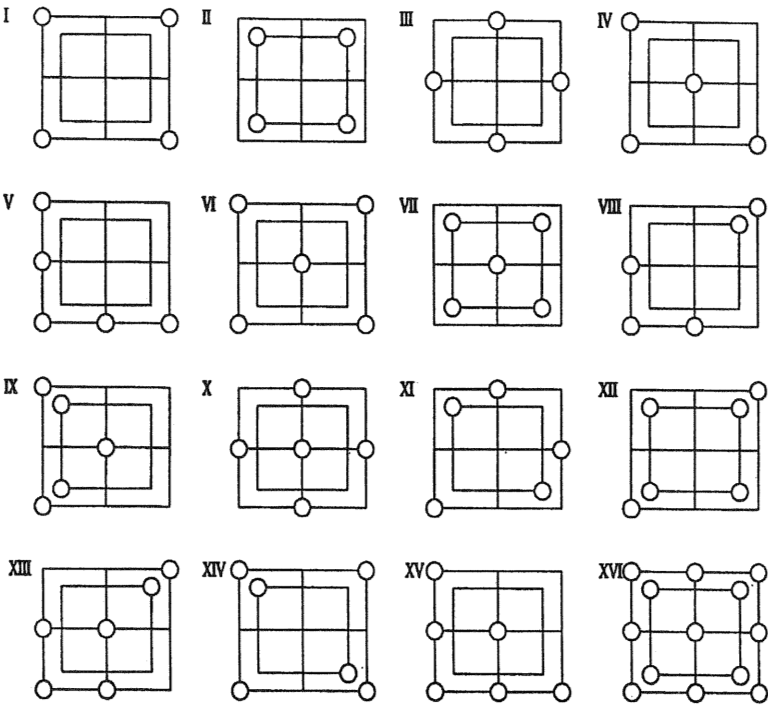


Figure 8.4 Some experimental designs used in hybrid MLC. The abscises represent the concentration of surfactant, and the ordinates the concentration of modifier.

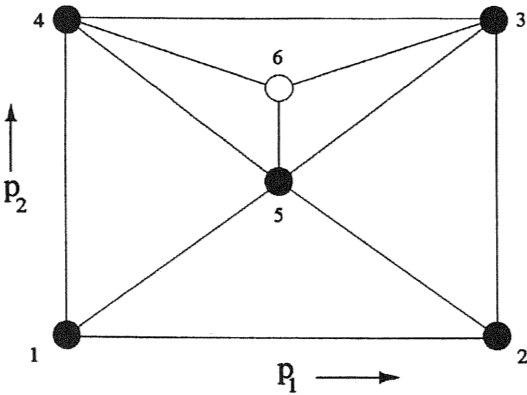


Figure 8.5 Method of triangles.

points and the central point (Fig. 8.5). For this reason, it will be called here method of triangles. The authors assumed that the retention of solutes was linearly related to the mobile phase variables within a selected portion of the space, and fitted a separate logarithmic linear function:

$$\log k = c_0 + c_1 [M] + c_2 \varphi \quad (8.15)$$

in each triangular subspace. The method was applied to the optimization of the separation of a set of fifteen phenols eluted with hexadecyltrimethylammonium bromide (CTAB) and 2-propanol at pH 7, and thirteen amino acids and small peptides eluted with SDS and 2-propanol at pH 2.5. Torres Lapasió et al. [10] later used, instead of the logarithmic function, a hyperbolic dependence.

The method of triangles can be considered as intermediate between a true sequential method and an interpretive method. The retention in other mobile phases is calculated by linear interpolation inside each triangle where the coordinates belong. Further, a chromatogram is simulated and compared with experimental data to verify the quality of the prediction. When experimental and predicted chromatograms coincide, a confirmation of the assumed linearity is obtained. However, when strong deviations of the linear model are observed, additional data-points should be included to refine the prediction, and this is usually performed without leaving the region of the variable space initially chosen, by a further subdivision of the response surface into smaller triangles (see Fig. 8.5). This, obviously, can result in an undesirable large number of experiments, and can cause the elimination of significant maxima in the first steps of the optimization process. If the optimum is found near one of the experimental points of the design, the prediction will be reliable. On the contrary, if it is at the center of one triangle subspace, serious errors may result.

Certainly, the success or failure of the method of triangles depends on the correctness of the linearity assumption of the retention model. The use of the logarithm of a function instead of the function to make a linear interpolation is a common practice, when the range of variation of the function must be reduced. The division of the variable space into four triangles reduces even more the variations of the function (*i.e.*, a function

that does not fit in the whole variable space may be acceptable over a smaller range). However, it has been demonstrated that the use of a hyperbolic function makes less necessary the addition of new mobile phases to refine the predictions, due to the better fitting accuracy [10].

III.3. Empirical Equations describing the Whole Variable Space

The scheme of interpolation followed in the iterative regression strategy explained above is not very simple from a practical point of view. The method of triangles requires at least four different equations, one for each established subspace. The use of a single equation to describe the retention behavior of a solute, in the whole variable space, seems to be more convenient to predict the retention of a solute in any mobile phase, with a minimum effort. Table 8.1 shows some of the models (equations) that have been considered, where the logarithm (eqs. 8.15-8.22), or the reciprocal of the retention factor (eqs. 8.23-8.30) are related to micelle concentration and volume fraction of organic modifier.

To obtain an adequate equation, a detailed examination of the retention behavior is required. Figure 8.6 shows plots of the reciprocal of the retention factor of noradrenaline vs. SDS concentration for constant 1-propanol concentration, and vs. 1-propanol concentration for constant SDS concentration [9]. Good agreement between experimental and calculated data is observed. The retention factor of noradrenaline decreases at increasing 1-propanol volume fraction, for each SDS concentration studied (Fig. 8.6b). However, this effect is attenuated as the concentration of surfactant increases, that is, the elution strength of 1-propanol decreases at increasing concentration of surfactant. The same behavior is observed with the surfactant (Fig. 8.6a) (i.e., its elution strength decreases at increasing concentration of modifier). Thus, noradrenaline showed a similar variation of the retention factors with respect to both surfactant and modifier.

After observing these results, Torres Lapasió et al. [9] decided to make a more extensive study of the capability of the empirical equations given in Table 8.1, to describe the retention behavior at any surfactant and modifier concentration. The errors in the prediction of the retention for a set

Table 8.1 Relative Global Errors (%) in the Prediction of Retention Factors for Five Catecholamines (Noradrenaline, Adrenaline, Adrenalone, Dopamine, and Isoprenaline), Eluted with Mobile Phases of SDS (0.035-0.15 M) and 1-Propanol (0-10%) at pH 6.8, Using Several Equations and Experimental Designs [9, 11]

Models	Experimental designs ^a															
Logarithmic equations	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
(8.15) $\log k = c_0 + c_1 [M] + c_2 \varphi$	20.5	11.3	12.8	17.9	19.9	17.6	13.6	25.3	45.9	15.4	32.2	35.6	25.0	17.6	20.8	20.6
(8.16) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]^2$	25.0	12.2	9.7	9.9	11.7	10.1	8.0	25.4	>50	10.4	17.6	22.7	24.4	18.0	11.7	13.3
(8.17) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 \varphi^2$	18.5	15.4	11.8	12.3	16.5	12.5	9.5	36.4	>50	14.6	18.0	21.8	34.7	13.8	16.7	29.6
(8.18) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi$	22.1	11.0	22.5	27.2	38.1	24.4	13.8	13.6	33.4	21.7	33.2	24.1	16.2	22.6	24.8	16.1
(8.19) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 [M]^2$	- ^b	- ^b	- ^b	- ^b	>50	8.4	7.9	11.8	>50	26.6	18.7	21.2	13.1	9.9	11.5	10.6
(8.20) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 \varphi^2$	- ^b	- ^b	- ^b	- ^b	103	10.6	9.3	16.6	>50	29.5	24.6	23.3	22.4	12.1	23.7	14.1
(8.21) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 [M]\varphi^{1/2}$	- ^b	- ^b	- ^b	- ^b	40.5	13.1	25.9	13.6	>50	23.3	>50	>50	14.7	13.8	29.4	14.9
(8.22) $\log k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 \varphi [M]^{1/2}$	- ^b	- ^b	- ^b	- ^b	37.9	17.4	11.5	13.4	>50	>50	>50	32.4	14.1	15.0	25.3	13.8
Hyperbolic equations																
(8.23) $1/k = c_0 + c_1 [M] + c_2 \varphi$	25.3	17.4	>50	23.1	32.3	20.3	17.5	25.5	17.3	>50	17.3	17.1	22.7	21.2	28.2	20.4
(8.24) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]^2$	29.1	16.4	>50	23.9	32.2	20.8	38.9	32.3	14.2	>50	15.8	15.3	27.3	20.6	29.2	20.3
(8.25) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 \varphi^2$	24.8	19.8	39.5	24.4	31.7	21.6	38.9	35.1	19.3	44.1	15.1	15.1	29.5	21.5	28.0	20.0
(8.26) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi$	3.4	4.9	9.4	3.3	28.9	3.3	4.7	3.7	5.4	9.2	4.8	4.5	3.6	3.6	3.5	3.9
(8.27) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 [M]^2$	- ^b	- ^b	- ^b	- ^b	>50	3.4	5.2	32.3	5.4	7.4	5.0	4.8	4.0	3.7	3.4	3.8
(8.28) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 \varphi^2$	- ^b	- ^b	- ^b	- ^b	26.2	3.1	6.9	>50	5.3	25.0	4.0	3.8	3.9	3.4	3.1	3.5
(8.29) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 [M]\varphi^{1/2}$	- ^b	- ^b	- ^b	- ^b	43.5	2.8	9.8	12.8	5.5	8.9	3.4	3.1	3.5	2.8	4.1	2.5
(8.30) $1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M]\varphi + c_4 \varphi [M]^{1/2}$	- ^b	- ^b	- ^b	- ^b	29.2	3.4	5.2	6.2	5.7	32.3	5.7	8.0	3.7	3.9	3.5	3.9

^a Experimental designs shown in Fig. 8.4.

^b Insufficient experimental points for the fitting.

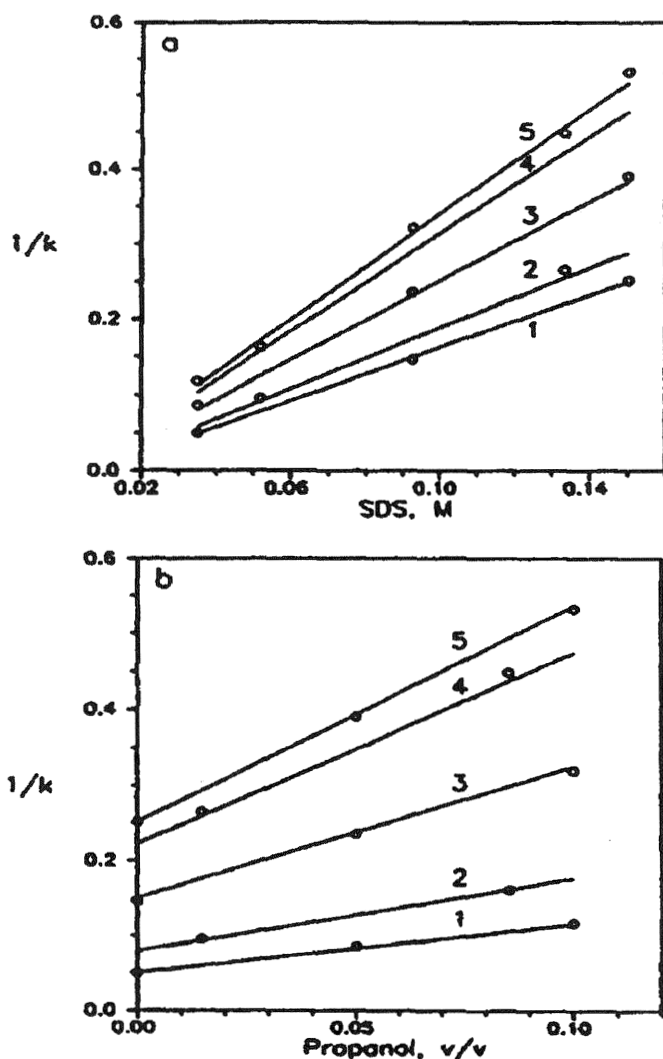


Figure 8.6 Retention behavior for noradrenaline: (a) $1/k$ vs. total concentration of surfactant plot for: (1) 0, (2) 0.015, (3) 0.05, (4) 0.085, and (5) 0.10 (v/v) 1-propanol; (b) $1/k$ vs ϕ plot for: (1) 0.035, (2) 0.052, (3) 0.092, (4) 0.133, and (5) 0.150 M SDS. Solid lines represent theoretical curves obtained from eq. 8.26; circles correspond to experimental k values. Reprinted from Ref. 9 with permission of Elsevier.

of five catecholamines were evaluated, using several models and more than one hundred experimental designs, some of them represented in Fig. 8.4.

The results given by the logarithmic equations are poorer. These functions systematically give larger k values [10]. Equation 8.23 also gives a bad prediction of the retention when used to describe the whole variable space. However, this simplified equation yields acceptable results in a small region of the variable space, as when applied in the method of triangles. The smallest errors were obtained with eqs. 8.26-8.30 [9,11]. It is thus evident that a term including both concentrations of surfactant and modifier is needed to model the retention.

The most simple equation giving acceptable results is eq. 8.26. It has been checked that the prediction capability of this equation is similar to the other more complex (eq. 8.27-8.30), for polar or moderately nonpolar compounds, such as amino acids [12], sulfonamides [13], β -blockers [14], and diuretics [15] (C18 column and mobile phases of SDS and 1-propanol or 1-pentanol), and some benzene and naphthalene derivatives (C8 or C18 columns and mobile phases of SDS or CTAB, and 1-propanol or 1-butanol) [9,16]. Thus, for these compounds, the plot of the reciprocal of k vs. concentration of modifier is linear at a fixed surfactant concentration. It is important to remark that the accuracy of the model has been checked with data obtained by several authors.

Further, García et al. [16] found that changes in the concentration of surfactant and alcohol, in the mobile phase, influence the relative errors obtained with eq. 8.26, which were lower for CTAB with respect to SDS, and for 1-propanol with respect to 1-butanol. This simple model failed in taking into account some interactions of the solutes, which are more important in very hydrophobic systems and when solute-micelle interactions are diminished. It should be considered that the amount of surfactant on the column desorbed by 1-butanol is greater than by 1-propanol. Also, 1-butanol can compete to a greater extent than 1-propanol with the micelle, in the interaction with the solutes. Eq. 8.28 (with a ϕ^2 term) provided a better description of the retention for highly hydrophobic polycyclic aromatic hydrocarbons (PAHs) than eq. 8.26 (Fig. 8.7). For these compounds, the plot of $1/k$ vs. ϕ was nonlinear. Similar results were obtained for several steroids eluted with SDS-acetonitrile eluents [17].

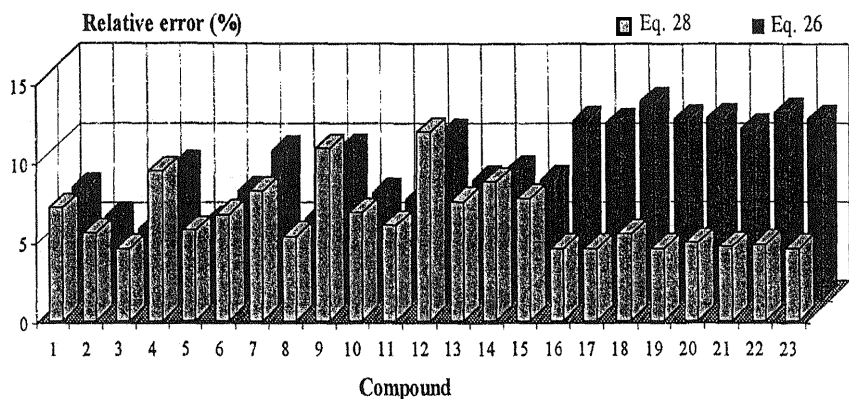


Figure 8.7 Relative errors (%) obtained in SDS-1-butanol mobile phases, for the retention factor prediction of: (1) benzene, (2) benzylic alcohol, (3) benzamide, (4) toluene, (5) benzonitrile, (6) nitrobenzene, (7) phenol, (8) 2-phenylethanol, (9) chlorobenzene, (10) phenylacetonitrile, (11) 3,5-dimethylphenol, (12) naphthalene, (13) 1-naphthol, (14) 2-naphthol, (15) 1-naphthylamine, (16) pyrene, (17) phenanthrene, (18) 2,3-benzofluorene, (19) fluorene, (20) fluoranthene, (21) acenaphthylene, (22) acenaphthene, and (23) anthracene. Adapted from Ref. 16.

Table 8.1 shows that the addition of a $[M]\varphi^{1/2}$ term (eq. 8.29) improves the accuracy of the predictions for some experimental designs. Although experimental designs of four, five and six points, such as designs I, VI and XIV in Fig. 8.4, are enough to achieve the fitting parameters of eqs. 8.26, 8.28 and 8.29, respectively, an additional measurement should be used at least to check the accuracy of the fittings.

Calculated k values according to eq. 8.26 and design VI (Fig. 8.4) are plotted in Fig. 8.8, against experimental values, for: (i) five catecholamines (noradrenaline, adrenaline, adrenalone, dopamine, and isoprenaline) and thirteen mobile phases (0.035-0.150 M SDS and 0-15% 1-propanol), (ii) fifteen phenols (4-benzamidephenol, 4-hydroxy-benzyl alcohol, 4-hydroxyphenemethyl alcohol, 4-hydroxybenzyl cyanide, 4-hydroxyacetophenone, 4-hydroxybenzaldehyde, phenol, 4-fluorophenol, 4-

hydroxypropiofenone, 4-methylphenol, 4-nitrophenol, 4-hydroxybenzophenone, 4-isopropylphenol, 4-hydroxydiphenylmethane, 4-*tert.*-butylphenol) and five mobile phases (0.04-0.12 M CTAB and 0-10% 2-propanol), (iii) thirteen amino acids and small peptides (arginine, methionine, tryptophan, tyrosine, alanyl-tyrosine, arginyl-phenylalanine, aspartyl-phenylalanine, leucyl-tryptophan, leucyl-tyrosine, lysyl-phenylalanine, phenylalanyl-phenylalanine, glycyl-leucyl-tyrosine, and glycyl-phenylalanyl-leucine) and five mobile phases (0.1-0.4 M SDS and 0-15% 2-propanol), and (iv) six aromatic compounds (anisole, benzene, naphthalene, 1-naphthalenemethanol, phenol and toluene) and fifteen mobile phases (0.06-0.14 M SDS and 10% 1-propanol) [9]. The equations for the fitted straight-lines (linear least squares) were $k_{\text{calc}} = 0.22 + 0.98 k_{\text{exp}}$ ($r = 0.998$) for the catecholamines, $k_{\text{calc}} = 0.05 + 1.00 k_{\text{exp}}$ ($r = 0.998$) for the phenols, $k_{\text{calc}} = -0.41 + 1.05 k_{\text{exp}}$ ($r = 0.9996$) for the amino acids and peptides, and $k_{\text{calc}} = 0.24 + 0.99 k_{\text{exp}}$ ($r = 0.996$) for the diverse aromatic compounds. The proximity of the slope to unity and the low intercept revealed the absence of systematic errors.

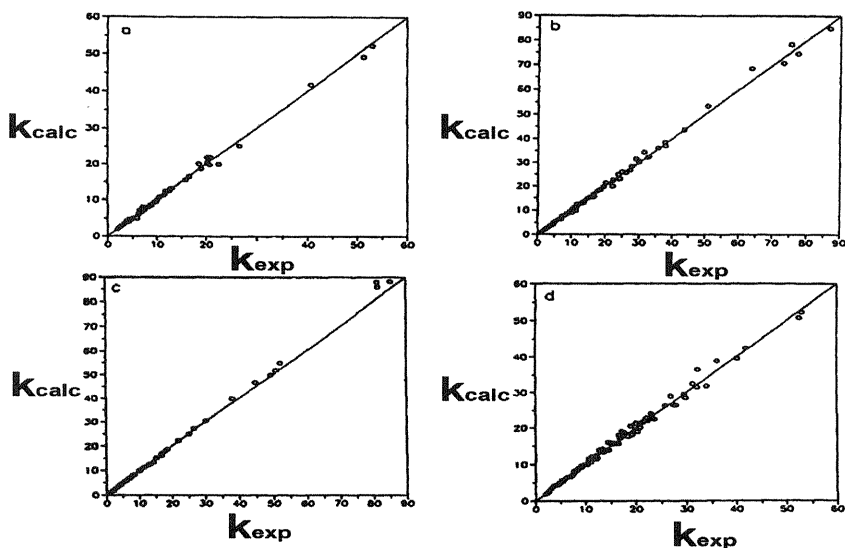


Figure 8.8 Calculated k vs. experimental k values according to eq. 8.26 and design VI (see Fig. 8.4) for: (a) five catecholamines and thirteen mobile phases, (b) fifteen phenols and five mobile phases, (c) thirteen amino acids and peptides and five mobile phases, and (d) six aromatic compounds and fifteen mobile phases. Reprinted from Ref. 9 with permission of Elsevier.

The prediction of retention factors for mobile phases showing the smallest elution strength, such as pure micellar solutions with a concentration of surfactant close to the cmc, is often very poor, especially for nonpolar solutes. It must be considered that for these mobile phases, an extrapolation is performed in a region of strong change in k , where small variations in the concentrations of surfactant and modifier affect highly the retention. Finally, the accuracy of the model for each component in a mixture can be variable, and the degree of tolerable variability for any component in a given separation is dependent upon how important that particular component is to the overall quality of the separation.

III.4. Prediction of the Retention and Selectivity

The isolines in Fig. 8.9 represent mobile phase compositions where the same retention is expected for 4-nitrophenol, as predicted from the retention data of mobile phases of 0.04 M CTAB, 0.04 M CTAB-10% 2-propanol, 0.08 M CTAB-5% 2-propanol, 0.12 M CTAB, and 0.12 M CTAB-10% 2-propanol. It is obvious that when both micelle and modifier concentrations are increased, the respective effects are combined and an even shorter retention is observed. In this way, the retention decreases from a retention factor of 63.8 in the lower left corner of the diagram to 10.2 in the upper right corner.

Although the above trend is observed for all components in a mixture, it must be stressed that the amount of reduction will not be the same, thus resulting in changes in the selectivity coefficient, α , which is defined as the ratio of the retention factors of two components, where numerator and denominator are selected such that the resulting value is larger than 1. Fig. 8.10 (top) shows the effect of varying surfactant and alcohol concentration on the selectivity of phenol and 4-hydroxybenzaldehyde: the selectivity is scarcely affected by 2-propanol, due to a similar change in retention of both compounds. However, by increasing the surfactant concentration, an increase in selectivity is observed due to the fact that 4-hydroxybenzaldehyde shows a stronger drop in retention than phenol. The final separation in 0.12 M CTAB is clearly superior to the ones observed at other mobile phase compositions. The reverse is observed when the retention behavior of 4-hydroxypropiophenone

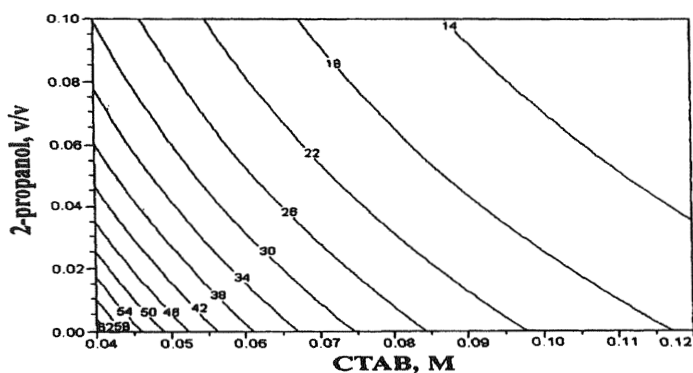


Figure 8.9 Retention factor of 4-nitrophenol as a function of surfactant and modifier concentration. The lines connect points defined by equal retention factors.

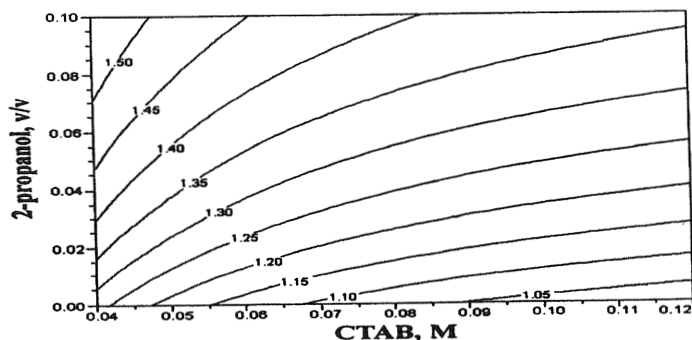
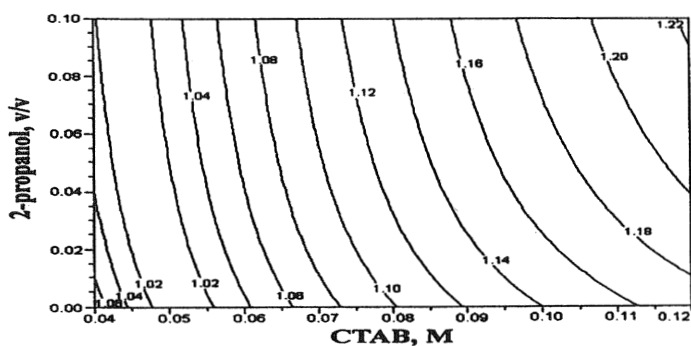


Figure 8.10 Selectivity, α , of: phenol and 4-hydroxybenzaldehyde (top), and 4-hydroxypropiophenone and 4-nitrophenol (bottom), as a function of surfactant and alcohol concentration. The lines connect points of identical selectivity.

and 4-nitrophenol is examined (Fig. 8.10, bottom): a reduction in selectivity occurs when the surfactant concentration is increased, and the two components coelute in 0.12 M CTAB. In contrast, when the alcohol concentration is increased from 0 to 10% (v/v), the selectivity increases, since the reduction in retention of 4-hydroxypropiophenone is stronger than the change in retention of 4-nitrophenol.

A much stronger change in retention of a compound in a mixture than the surrounding components, with varying conditions, causes numerous cases of coelution and peak-crossings in intermediate mobile phase compositions. Therefore, a satisfactory separation can only be found through a systematic search of the variable space.

IV. PHYSICOCHEMICAL MEANING OF THE EMPIRICAL MODELS

The equations that describe the retention on hybrid micellar mobile phases were first derived on a pure empirical basis. A further concern was to find an interpretation of these equations, based on physico-chemical properties. This permitted the improvement of the descriptive models, and the evaluation of the parameters of interaction between the three environments involved in MLC: stationary phase, bulk water, and micelles, according to equilibria 8.1 and 8.2 [18]. The coefficients in eq. 8.26 were related to several parameters of retention. From the reciprocal of this equation:

$$k = \frac{1}{c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi} \quad (8.31)$$

an expression similar to the reciprocal of eq. 8.3 can be obtained:

$$k = \frac{K_{AS} \frac{1}{1 + K_{AD}\varphi}}{1 + K_{AM} \frac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi} [M]} \quad (8.32)$$

being:

$$K_{AS} = \frac{1}{c_0} ; K_{AM} = \frac{c_1}{c_0} ; K_{AD} = \frac{c_2}{c_0} ; K_{MD} = \frac{c_3}{c_1}$$

This equation can be rewritten as:

$$k = \frac{K_{AS}^{\phi}}{1 + K_{AM}^{\phi} [M]} \quad (8.33)$$

where K_{AS}^{ϕ} and K_{AM}^{ϕ} are apparent constants with respect to the concentration of modifier. It was observed however that the convergence of the iterative process in the nonlinear regression was more rapid and stable when the experimental data were fitted to eq. 8.31 instead of eq. 8.32. In eq. 8.32, the constants K_{AD} and K_{MD} measure the relative variation in the concentration of solute in bulk water and micelle, respectively, in the presence of modifier taking the pure micellar solution (without modifier) as the reference:

$$K_{AS}^{\phi} = \frac{[AS]}{[A] + \Delta[A]} = K_{AS} \frac{1}{1 + K_{AD} \phi} \quad (8.34)$$

$$K_{AM}^{\phi} = \frac{[AM] + \Delta[AM]}{[M] ([A] + \Delta[A])} = K_{AM} \frac{1 + K_{MD} \phi}{1 + K_{AD} \phi} \quad (8.35)$$

where $[A]$ and $[AM]$ are the concentrations of free solute in bulk water and solute associated to the micelle in a pure micellar solution, and $\Delta[A]$ and $\Delta[AM]$ are the changes in the concentrations produced by the modifier.

On the other hand, eq. 8.28 can be rearranged as follows:

$$k = \frac{K_{AS} \frac{1}{1 + K_{AD} \varphi + K \varphi^2}}{1 + K_{AM} \frac{1 + K_{MD} \varphi}{1 + K_{AD} \varphi + K \varphi^2}} [M] \quad (8.36)$$

This equation introduces a new constant, K , that implies a quadratic hyperbolic variation in K_{AS} and K_{AM} with φ , which may suppose an excessive dependence of the retention with φ , and produce high errors when an extrapolation is made in a region of large concentrations of modifier. Therefore, an alternative model was proposed for highly hydrophobic solutes, which considers the additional change in the concentration of solute associated to the stationary phase, produced by the presence of modifier [18]:

$$k = \frac{K_{AS} \frac{1 + K_{SD} \varphi}{1 + K_{AD} \varphi}}{1 + K_{AM} \frac{1 + K_{MD} \varphi}{1 + K_{AD} \varphi}} [M] \quad (8.37)$$

This equation is an extension of eq. 8.32, and has been checked to give a significant improvement in the prediction of the retention of highly hydrophobic solutes, such as pyrene.

The constants K_{MD} and K_{AD} in eq. 8.37 account for the displacement of the water-micelle equilibrium, whereas K_{SD} and K_{AD} describe the modification of the water-stationary phase equilibrium (see Fig. 8.1). These changes are due to the diminution in the polarity of water, and the modification of the interactions of the solute with micelles and stationary phase, when a modifier is added.

V. SIMULTANEOUS EFFECT OF pH, MICELLE AND ORGANIC MODIFIER

V.1. *Iterative Regression Strategy*

Although two variables usually suffice for samples of moderate complexity to obtain a satisfactory separation, inclusion of a third variable will often further improve the quality of the separation, with respect to both resolution and analysis time. In the first methods developed in MLC for the analysis of compounds showing an acid-base behavior, the pH was usually previously chosen and only the concentrations of surfactant and modifier were optimized. The best pH for the separation was selected after examining the retention in a reduced number of mobile phases, at two or three pH values. However, for an adequate method development, this variable should be simultaneously optimized with the concentrations of surfactant and modifier, especially because the protonation constants of solutes suffer shifts, depending on the composition of the mobile phase. This is caused by the different partitioning of the acidic and basic species of solutes in the micellar pseudophase, due to electrostatic interactions.

Strasters et al. [19] were again concerned with this problem, and considered the simultaneous optimization of pH, and concentrations of surfactant and modifier, for the separation of a mixture of several amino acids and peptides. For this purpose, the authors used the method of triangles adapted to three dimensions, and linear functions of $\log k$ vs. pH, surfactant and modifier. The procedure began with a design of fifteen points located in a three-dimensional space, which was divided into 24 tetrahedra (Fig. 8.11). Therefore, 24 different equations of retention should be fitted. The retention in other mobile phases was calculated by linear interpolation inside each tetrahedron. The range of pH values examined by the authors for the prediction of the retention was intentionally reduced to pH 2.5-3.5, to prevent deviations from linearity in the retention behavior of the amino acids and peptides. This was, obviously, a great limitation of the procedure.

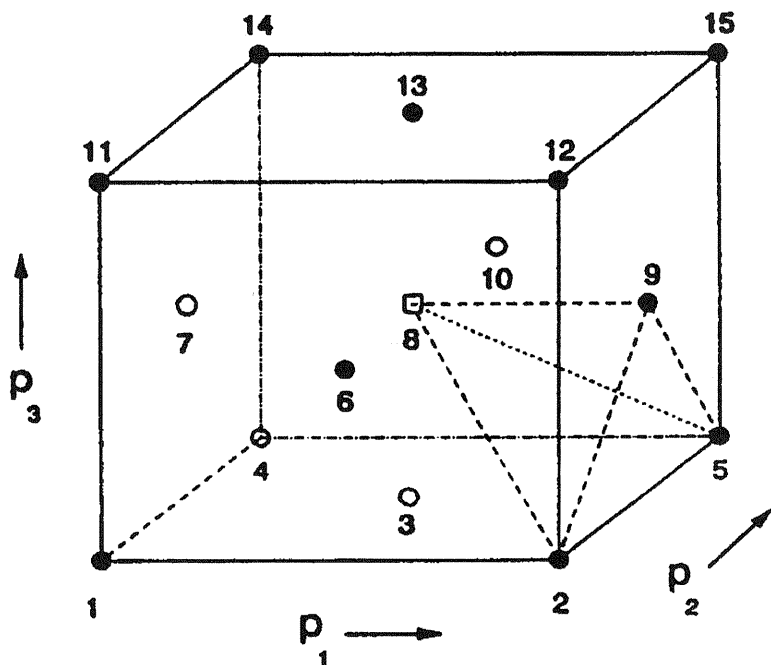


Figure 8.11 Initial experiments in a three-parameter iterative regression optimization. The solid dots represent points located on the visible outside of the cube, the open dots (3, 4, 7 and 10) are measurements on the invisible sides of the cube, and the open square (8) is located in the centre of the cube. One of the subspaces, the tetrahedron (2, 5, 8, 9) is indicated by the dashed lines.

V.2. Global Model

A mathematical model was further developed [20], which extended the description made with eq. 8.32 to take into account the influence of pH on retention. The effect of the modifier on the description of the retention can be considered by substitution, in eq. 8.11, of eqs. 8.34 and 8.35, and other similar changes for the protonated species. The following was obtained:

$$k = \frac{\frac{\frac{K_{AS}}{1 + K_{AD}\varphi}}{1 + K_{AM}\frac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi}[M]} + \frac{\frac{K_{HAS}}{1 + K_{HAD}\varphi}}{1 + K_{HAM}\frac{1 + K_{HMD}\varphi}{1 + K_{HAD}\varphi}[M]} \kappa K_H h}{1 + \kappa K_H h} \quad (8.38)$$

where:

$$\kappa = \frac{1 + K_{HAM}\frac{1 + K_{HMD}\varphi}{1 + K_{HAD}\varphi}[M]}{1 + K_{AM}\frac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi}[M]}$$

Eq. 8.38 may be rewritten as:

$$k = \frac{k_A + k_{HA} K_H^{M\varphi} h}{1 + K_H^{M\varphi} h} \quad (8.39)$$

where $K_H^{M\varphi}$ is the apparent protonation constant of the solute, that depends on the concentration of both surfactant and modifier and on the association capability of both acid-base species with the micelle. The modifier decreases $K_H^{M\varphi}$, whereas this constant increases slightly with the concentration of surfactant.

Equation 8.38 contains nine constants (K_{AS} , K_{AM} , K_{AD} , K_{MD} , K_{HAS} , K_{HAM} , K_{HAD} , K_{HMD} and K_H), and describes the change in the retention factors of acid-base solutes at any pH, and any concentration of surfactant and modifier in the mobile phase. However, the model does not consider the modification of K_H in the water-modifier bulk solvent, due to the change in the concentration of modifier. A more complete model would require the

introduction of a new constant. It was checked, however, that the inclusion of this constant does not improve significantly the description of the retention, since the large number of parameters of the model provides a high flexibility to the fitting, absorbing the deviations produced by this simplification.

Partial fittings of the data can be made to obtain initial values that facilitate the rapid and reliable convergence of eq. 8.38 towards the correct solution, and avoid local minima. Thus, the retention factors in four mobile phases at pH 7 could be used to calculate the four constants of the nonprotonated species (eq. 8.32). The parameters of the protonated species could be obtained, similarly, at a sufficiently acid medium, whereas the estimation of the protonation constant will require additional mobile phases at intermediate pH values. Using these initial parameters, the global nonlinear fitting of the complete set of experimental data can be made more easily.

The determination of the parameters of the model given by eq. 8.38 requires experimental data from at least nine mobile phases, but extra data should be used to improve the reliability of the predictions. The model was successfully applied to the prediction of the retention behavior of seven solutes (benzocaine, bumetanide, ethacrynic acid, furosemide, sulfanilamide, tyrosine and xipamide) inside the total pH range of a C18 column, and for the concentration ranges of SDS and 1-propanol, 0.05-0.15 M and 0-8% (v/v), respectively, with errors lower than 6% [20].

V.3. *Separation of Amino Acids*

The importance of performing a simultaneous optimization of the three variables, pH, and concentrations of micelles and modifier, is illustrated by the separation of a mixture of amino acids and small peptides (Fig. 8.12) [19]. Apparently, a good separation is given at pH 2.5 for 0.1 M SDS and 4% (v/v) 2-propanol. The resolution is more than adequate, but the analysis time is relatively long (*ca.* 40 min). The same applies to the optimum predicted at pH 3.5, where similar resolution and analysis time are observed at 0.14 M SDS and 1% 2-propanol. However, when the full variable space is taken into consideration, an even better separation is obtained. At pH 3.1,

0.24 M SDS and 2% 2-propanol, the optimum situation is obtained. Not only is the resolution improved (in fact, an unnecessary improvement) but, more important, the analysis time is drastically reduced to 20 min. Hence, two variables are sufficient to obtain a satisfactory separation for this mixture, but three variables give a better chromatogram with respect to both separation and analysis time.

However, the overall analysis time will be a function of pH only if the degree of dissociation of the latter components in the chromatograms change in the examined pH range. As a consequence, the pH can be used to fine tune the selectivity, without influencing the elution strength of the mobile phase.

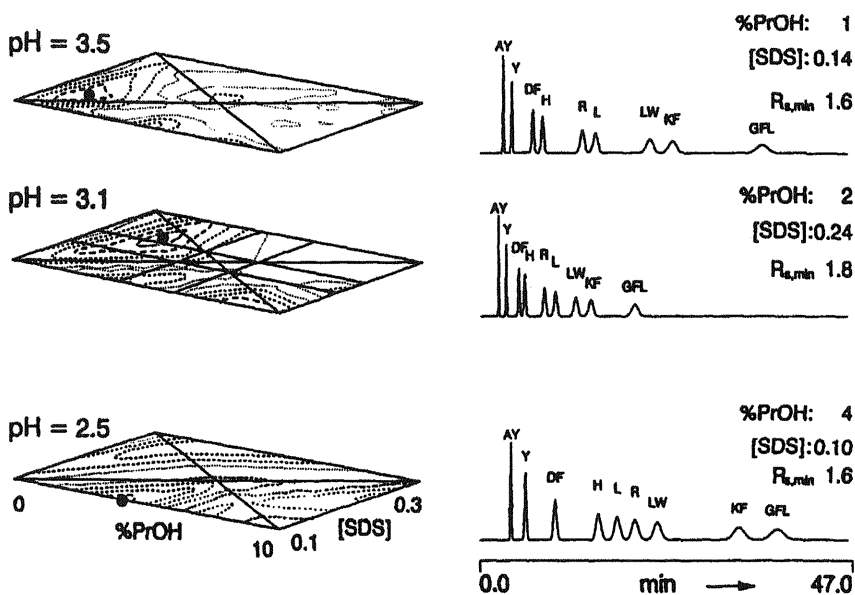


Figure 8.12 Three intersections in the original variable space at different pH values, for SDS and 2-propanol mobile phases used for the separation of a mixture of amino acids and small peptides. In each intersection, isoresponse lines show the behavior of the resolution criterion. The predicted optimum at each pH (indicated by the dot) is displayed on the right. The optimum at pH 3.1 is the predicted global optimum of the three-parameter optimization. Reprinted from Ref. 19 with permission of Elsevier.

VI. NEURAL NETWORKS

In recent years, neural networks have been widely used for solving different chemical problems, including the field of liquid chromatography, such as the modeling and prediction of the retention using structural descriptors, chromatographic peak classification and deconvolution of overlapped peaks. In MLC, Xie et al. [21] applied multi-layer feed-forward neural networks, trained with an error back-propagation algorithm, to model the retention behavior as a function of pH, concentrations of surfactant and organic modifier, and temperature. Other contributions came later [22, 23]. Accurate retention predictions were always achieved.

The soft models defined by the weights of the neural networks are capable of accommodating all types of relationships, being especially useful when the dependence of the retention behavior with the mobile phase variables is unknown. However, neural networks learn the relationships from the data themselves, and hence, more experimental points are needed with respect to hard-modeling methods. The use of neural networks is, therefore, only recommended for those cases where adequate theoretical or empirical models do not exist, such as retention modeling in MLC with four variables (e.g., pH, surfactant, modifier, and temperature).

VII. FACTORS AFFECTING THE PREDICTION CAPABILITY OF THE MODELS

VII.1. Linearization of the Equations of Retention

In the literature, linear regression has often been applied to fit the experimental data to the model that describes the dependence of the retention factor with the concentration of surfactant (eq. 8.3). However, the linearization process introduces a perturbation in the fitting. The least-squares method performs an implicit weighting of the experimental points, that is proportional to the sensitivity of the signal with respect to the variable being fitted. When an equation is linearized, the fitting data are modified, and consequently, the implicit weighting is perturbed [24]. A weighting strategy should be employed to diminish the systematic errors

produced with the linear transformation, and obtain output data similar to those given by nonlinear regression. The weights are given by:

$$W = \frac{\left(\frac{\delta f}{\delta P} \right)^n}{\left(\frac{\delta F}{\delta P} \right)^2} \quad (8.40)$$

where f and F are the nonlinear and linearized equations, respectively, and P is a parameter of the model [24]. Substitution in eq. 8.40 of eqs. 8.23-8.30, for $n = 2$, results in:

$$W = \frac{1}{k^4} \quad (8.41)$$

Fitting errors, in average 25% larger, have been obtained with the unweighted linearized model, compared to the weighted fitting [18].

The constant K_{AS} in eq. 8.3 is related to the retention in a mobile phase at the cmc. Therefore, its calculation requires an extrapolation in a region of large variation in k . When unweighted linear fitting is performed, the errors can lead to negative intercepts in the $1/k$ vs. $[M]$ plots. The application of weights corrects these errors, at least partially.

VII.2. Dead Time Measurement

The reliable calculation of retention factors requires the accurate evaluation of the dead time of the chromatographic system. This is not an easy task when a micellar mobile phase is used. Due to changes in the surfactant adsorbed layer, the shape, height and sign of the perturbations appearing at the head of the chromatograms are unpredictable, especially when the nature of the injected solution is very different from that of the mobile phase. In the MLC literature, the dead time is usually measured by injection of water, aqueous solutions of NaNO_3 , NaI and KI , or organic solvents such as

methanol and acetonitrile. The criteria applied to locate the dead time are either the measurement of the position of the maximum of the first peak in the chromatograms, or the measurement of the time from the injection to the first deviation of the baseline. The first criterion is very simple, but the variability in the shape and position of the first peak with mobile phases of diverse composition yields, frequently, approximate values. In contrast, the start of the first main peak is fairly reproducible [25].

In general, it is questionable that the values given in the MLC literature were real dead times, although the measured times were probably close to them. It is implicitly assumed that the injected compound used to determine the dead time does not interact with the stationary phase. This is obviously not the case for methanol or acetonitrile that disrupt the adsorbed surfactant layer. It has been observed however that the time measured with the different salt solutions mentioned above is variable. More frequently, only a characteristic time in the head of the chromatograms, or reference time, will be determined. This time should be at least reproducible.

In fact, even the dead time measured by injection of water is not very reproducible, especially when the data are taken at the maximum of the first perturbation of the baseline. The measurement is better when performed by injection of micellar solutions of the analytes [25]. The experimental work can provide a great number of replicates which can be used for this purpose.

Figure 8.13 shows that when water is injected, two well differentiated regions appear at the beginning of the chromatogram. The first region is rather irregular and unpredictable, and the second region keeps its shape and position when the wavelength changes. On the other hand, the signals obtained by injection of a solution with a similar composition as the mobile phase (blank solution) keep the position of the maximum. In this case, only the height of the signal is modified, and the second perturbation is scarcely observed. The noise increase observed with the wavelength, when water is injected, is probably associated with the state of the detector lamp and of the material on the cell windows.

The first perturbation observed when water is injected has probably a refractometric origin, and the second one is an absorptiometric signal. The irregular shape of the first peak obtained with water is due to the large difference in composition between the injected solution and the mobile phase,

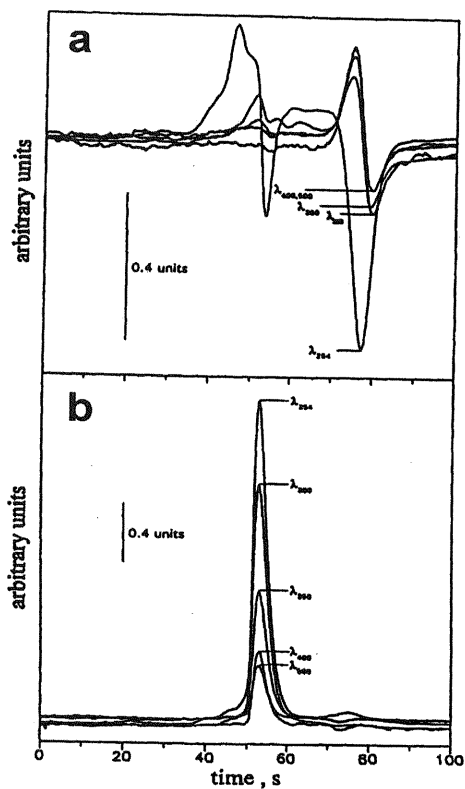
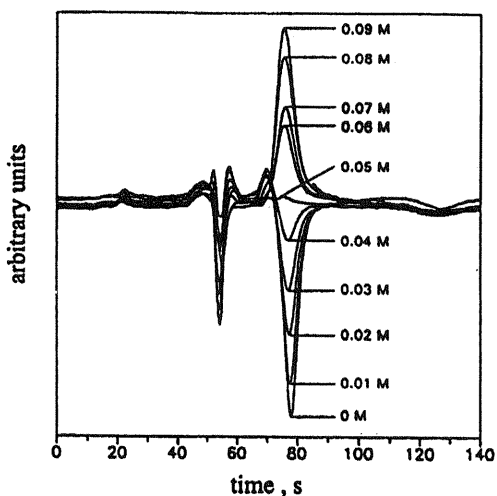


Figure 8.13 Influence of wavelength in the measurement of the reference time obtained by injection of: (a) micellar solution, and (b) water. A 0.05 M SDS mobile phase without modifier was used. Wavelengths are given in nm. Reprinted from Ref. 25.

Figure 8.14 Influence of SDS concentration in the injected solution on the shape of the signal at the beginning of the chromatogram, when a mobile phase of 0.05 M SDS without modifier is used. Reprinted from Ref. 25.



which originates erratic fluctuations in the refractive index when both solutions are mixed. In contrast, when the blank solution is injected, the homogenization of the mixture is more simple. Figure 8.14 shows another set of injections, where the concentration of SDS in the injected sample is varied from a value below the concentration of the mobile phase up to a concentration above it. It may be observed that when the compositions of both injected solution and mobile phase are matched, the second peak decreases and even disappears, and further, a positive signal is achieved. This behavior confirms the absorptiometric nature of this peak.

The reference time does not change appreciably with the composition of the eluent in hybrid mobile phases. Therefore, the same value can be used to predict the retention factors of solutes eluted in a given chromatographic column, with mobile phases containing variable amounts of surfactant and alcohol [18, 25]. Furthermore, an excellent prediction of retention times can be obtained using approximate values of the reference time, since the retention factor is only used as an intermediate variable in the prediction of the position of the chromatographic peaks. The evaluation of physicochemical retention coefficients requires, however, the use of an accurate value of the reference time. Otherwise, ill-conditioned fittings will give negative coefficients.

VII.3. Critical Micellar Concentration

Another important factor that should be considered, when physicochemical retention coefficients are calculated from chromatographic data in MLC, is the correct subtraction of the cmc from the total concentration of surfactant. The cmc of SDS solutions decreases at low concentrations of alcohols used as modifiers, except for methanol, and increases for acetonitrile and tetrahydrofuran. When the cmc is not subtracted from the total concentration of surfactant, the errors in the calculation of the retention coefficients can be larger than 100%. However, as occurs with the dead time, for many solutes there is no significant difference between the use of total or micellar concentration of surfactant in the prediction of the retention behavior (retention factors) [11, 18].

VIII. OPTIMIZATION OF THE RESOLUTION

The many interactions that the solutes experience in a micellar chromatographic system enhances the differences among them. The possibility of using, simultaneously, the three most significant variables that affect the retention (*i.e.*, pH, and concentrations of surfactant and modifier), will improve the capability of resolution of complex mixtures of ionic and nonionic compounds. The high accuracy in the prediction of retention factors in MLC permits the reliable and relatively rapid optimization of the composition of the mobile phase for the separation of a mixture of compounds, by using an interpretive method and a reduced number of mobile phases (at least two for one variable, four or five for two variables, and nine for three variables).

The optimization of the resolution of a mixture of compounds comprises several steps, as the following:

- (i) Achievement of the equations of retention for each solute.
- (ii) Search of the optimum mobile phase with the aid of maps of global resolution for the mixture of solutes.
- (iii) Simulation of the chromatogram for the optimum mobile phase.
- (iv) Search of a new maximum, when the selected optimum is not satisfactory.

The four criteria of resolution given below based on the normalized product, r , of different properties, $X_{i,i+1}$, associated to pairs of consecutive peaks, have been applied in MLC [8, 11, 26]:

$$r = \prod_{i=1}^{n-1} \frac{X_{i,i+1}}{\left(\sum \frac{X_{i,i+1}}{n-1} \right)^{n-1}} \quad (8.42)$$

The individual properties studied were the selectivities, separation factors, valley-to-peak ratios and overlapped fractions. The two first criteria only consider the position of the chromatographic peaks, and the latter two their position and shape. The three latter functions may vary from 0 to 1. The combined function of resolution, r , is maximized to obtain the optimum mobile phase, and its proximity to unity indicates the quality of the separation. Since the product of all observed resolutions is used, coelution will effectively cause the criterion to drop to zero. Extremely long chromatograms with a number of unnecessarily large resolution values will also be represented by low criterion values.

VIII.1. Strategies Strictly Based on Solute Retention

The most simple criteria used in chromatographic optimization are based on properties that only depend on the retention of solutes [11], such as the modified selectivity:

$$\beta_{i,i+1} = 1 - \frac{1}{\alpha_{i,i+1}} = 1 - \frac{k_{i+1}}{k_i} \quad (8.43)$$

and the separation factor:

$$S_{i,i+1} = \frac{t_{i+1} - t_i}{t_{i+1} + t_i} = \frac{k_{i+1} - k_i}{k_{i+1} + k_i + 2} \quad (8.44)$$

where $\alpha_{i,i+1}$ is the selectivity, and t_i , t_{i+1} , k_i , and k_{i+1} , are the retention times and retention factors of consecutive peaks.

The positional criteria can lead to reliable resolution optima using the retention data from a few mobile phases. However, as the shape and width of the chromatographic peaks are not considered, for largely overlapped asymmetric peaks, an unacceptable optimum can be obtained. If this is the case, only an increase in plate count will provide the desired separation, using for instance two identical columns in series [8]. Alternatively, a response surface related to a different criterion must be examined.

VIII.2. Strategies that Take into Account the Position and Peak Shape

The major drawback of practical separations applying MLC is still the low chromatographic efficiency, caused by the resistance to mass transfer in the processes involving micelles and a surfactant-modified stationary phase as exposed in Chapter 6. This is especially important since the increase in micelle concentration causes a decrease in plate count, resulting in a varying efficiency over the variable space. It is thus worthwhile to examine the inclusion of the expected peak shape in the expression of the chromatographic quality.

The information given by the positional-shape criteria is interesting, not only when the chromatographic peaks are asymmetric or have low efficiencies, but also with symmetric peaks which are very close to each other. Poorly defined optima obtained with a positional criterion often become clearer when the shape of the peaks is considered.

Two positional-shape criteria have been developed [11], the valley-to-peak ratio:

$$P_{i,i+1} = 1 - \frac{h_1}{h_2} \quad (8.45)$$

and the overlapped fractions:

$$O_i = 1 - \frac{w'_i}{w_i} \quad (8.46)$$

In these equations (see Fig. 8.15), h_1 is the height of the valley between two adjacent peaks, h_2 the interpolated height between the maxima of two adjacent peaks measured at the abscissa of the valley, w_i is the total area of a given peak, and w'_i the area of this peak overlapped by other peaks. The overlapped fractions extends the global function of resolution to all individual peaks in the chromatogram, $n-1$ should thus be substituted for n in eq. 8.42.

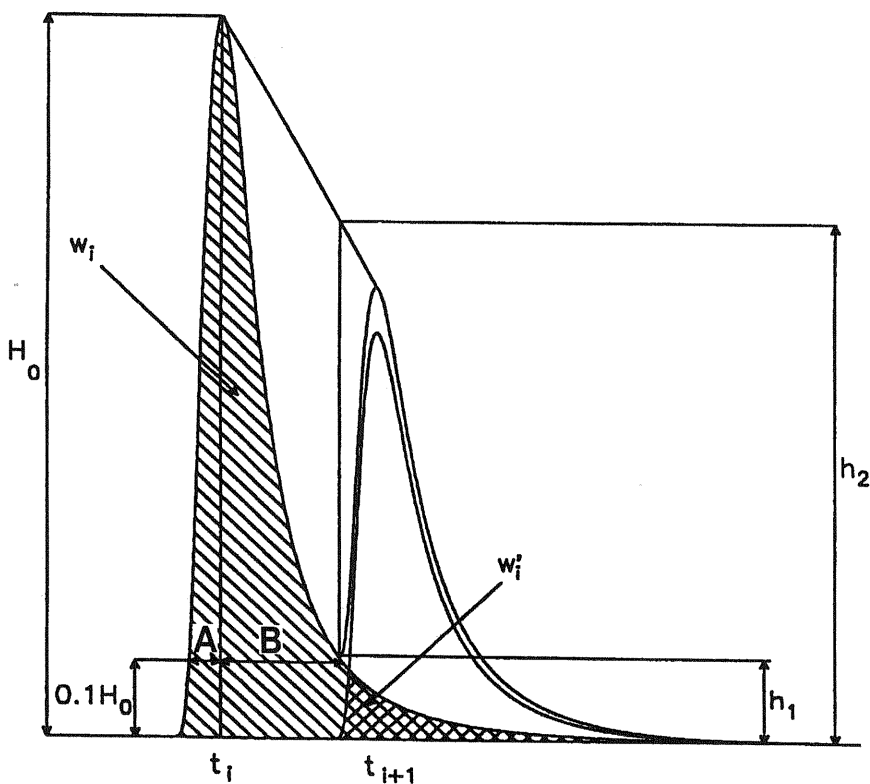


Figure 8.15 Chromatographic peak properties. Reprinted from Ref. 11 with permission of Elsevier.

VIII.3. Prediction of Peak Shape

The models used to predict peak shape, based on Gaussian distributions, have the advantage of using very intuitive parameters, related to properties which can directly be measured on the chromatograms (position and height of the maxima, and width of the peaks). The equation describing a pure Gaussian peak is:

$$h(t) = H_0 \exp \left[-\frac{1}{2} \left(\frac{t - t_R}{\sigma_G} \right)^2 \right] \quad (8.47)$$

where t is the time, H_0 and t_R are the height and time at the peak maximum, and σ_G the standard deviation. However, in MLC, skewed peaks with low efficiencies are often obtained, and in such cases, the assumption of a Gaussian model may yield large errors. A convenient modification of the Gaussian model is the substitution of the standard deviation in a pure Gaussian peak, by a polynomial function (polynomial modified Gaussian model, PMG), which varies with the distance to the maximum of the peak [27]:

$$h(t) = H_0 \exp \left[-\frac{1}{2} \left(\frac{t - t_R}{s_0 + s_1 (t - t_R) + s_2 (t - t_R)^2 + \dots} \right)^2 \right] \quad (8.48)$$

With this approach, accurate descriptions of peaks showing large asymmetries can be obtained, including those showing deformation either to the right or to the left. Also, eq. 8.48 with a linear or a parabolic function can be applied to refine peak parameters, such as the efficiency and asymmetry factor, estimated by direct measurement of the chromatographic signal. The method of Powell can be used to fit the experimental data to the nonlinear functions [28].

The coefficient s_0 in eq. 8.48 coincides with the standard deviation of a symmetric Gaussian peak describing the central region of the peak, and

remains almost constant when new terms are added to the polynomial function, whereas s_1 and s_2 are coefficients that quantify peak distortion. The coefficient s_1 only depends on the asymmetry factor; a large value of this coefficient indicates a strong asymmetry, and a negative value, a left bias; s_2 and upper terms in polynomials of higher degree correct small deviations to better fit the shape of the peaks. However, the use of a large number of s coefficients lessens the practical application of the model.

A linear standard deviation function in eq. 8.48 approximates satisfactorily the real shape of the peaks in a chromatogram, but the accuracy of the fitting can be easily improved by increasing the degree of the polynomial. The model with a linear function permitted, however, the development of a simple method to simulate chromatograms and to optimize the resolution of mixtures of compounds, using criteria that consider not only the position of the chromatographic peaks, but also their shape [27]. With the linear function, the number of chromatographic peak parameters (i.e., position, height, efficiency, and asymmetry factor) coincides with the number of coefficients in the $h(t)$ function (H_0 , t_R , s_0 and s_1). The coefficients s_0 and s_1 may be easily obtained from the asymmetry factor and efficiency. The steps to follow in the calculation of these coefficients, for the simulation of a peak in a chromatogram, are the following:

- (i) The retention time of a peak in a given mobile phase is predicted from the equations that describe the retention.
- (ii) The efficiency, N , and asymmetry factor, B/A , are estimated by interpolation after fitting to a plane the values of these parameters for experimental mobile phases close to the predicted mobile phase.
- (iii) The width of the peak at 10% of peak height is calculated from:

$$W_{0.1} = A + B = \sqrt{\frac{41.7 t_R^2}{N (1.25 + B/A)}} \quad (8.49)$$

and the individual values of A and B are obtained from $W_{0.1}$ and B/A :

$$A = \frac{W_{0.1}}{1 + B/A} \quad (8.50)$$

$$B = \frac{W_{0.1}}{1 + \frac{1}{B/A}} \quad (8.51)$$

- (iv) The height of the normalized peak can be determined by assuming a triangular profile: $H = E/W_{0.1}$, being E a normalization constant. If a higher precision is desired, other functions can be used. In the simulation of a real chromatogram, the normalization step can be obviated.
- (v) Finally, the coefficients s_0 and s_1 of the standard deviation of the skewed peak are calculated from eq. 8.48, for $t = t_R - A$, and $t = t_R + B$, making $h(t) = 0.1 H_0$. After solving the system of two equations, the following is obtained:

$$s_1 = \frac{1}{\sqrt{2 \ln 10}} \frac{B/A - 1}{B/A + 1} \approx 0.466 \frac{B/A - 1}{B/A + 1} \quad (8.52)$$

$$s_0 = \frac{B(1 - s_1 \sqrt{2 \ln 10})}{\sqrt{2 \ln 10}} \approx 0.466 B(1 - 2.146 s_1) \quad (8.53)$$

VIII.4. Contour Maps of Resolution

Different regions of the variable space will often be associated with different critical peak-pairs. The resolution of a multicomponent mixture thus requires an analysis involving all components in the whole variable space. Inspection of the contour maps of global resolution will allow the evaluation of the robustness of the optimum. Figure 8.16 shows the contour maps for the separation of a set of fifteen phenols (the same cited previously in this chapter), with mobile phases of CTAB and 2-propanol, where an efficiency $N = 2500$ was considered for all solutes. For the positional criterion (separation factor), the optimum was found for a mobile phase of 0.12 M CTAB-10% 2-propanol (Fig. 8.16a, upper corner of the variable space), whereas for the valley-to-peak criterion it was 0.102 M CTAB-10% 2-propanol (Fig. 8.16b), and for the overlapped fractions, 0.107 M CTAB-10% 2-propanol (Fig. 8.16c).

The simulated chromatograms for 10% 2-propanol and three different concentrations of surfactant are given in Fig. 8.17. The disagreement among the positional and positional-shape criteria is due to the retention behavior of peaks 13-15. As the concentration of surfactant decreased from 0.12 to 0.10 M (Fig. 8.17a and 8.17b), the valley-to-peak ratio improved, and the overlapped fractions decreased to a lesser extent. The positional resolution however became worse. A further reduction in the concentration of CTAB decreased both the positional and positional-shape resolution (see peaks 9-10 and 13-15).

The combined use of the diverse optimization criteria may give complementary information for selecting the optimum mobile phase. The positional criterion gives a rough approximation of the region where the peaks will be separated, but it does not evaluate accurately the quality of the separation. The valley-to-peak ratio indicates the region where the peaks will be apparent. Finally, the overlapped fractions show the region where the peaks will be better quantified, because a larger surface of each peak will be exposed. As the application of the positional-shape criteria requires a good prediction of the position and shape of the chromatographic peaks, they are in principle more susceptible to errors. However, these criteria are always preferable, even when asymmetric peaks are assumed to be symmetric. The most satisfactory optima should be observed in the contour maps of the three

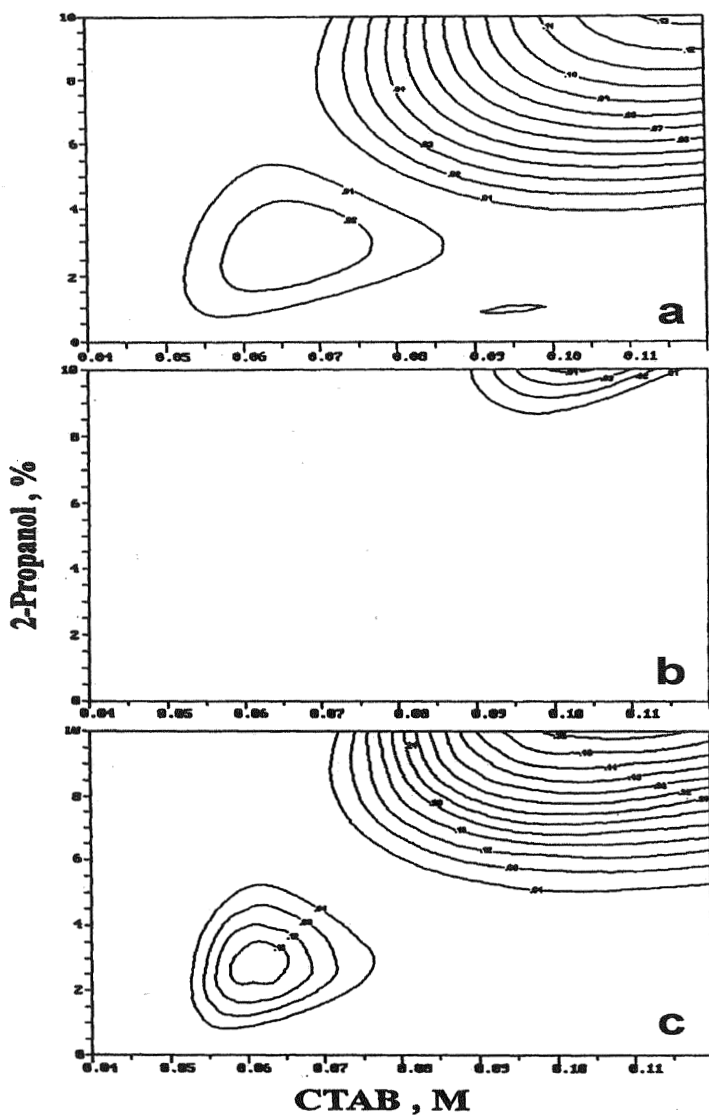


Figure 8.16 Contour maps of global resolution for the mixture of fifteen phenols eluted with CTAB-2-propanol mobile phases, according to different criteria: (a) separation factor, (b) valley-to-peak ratio, and (c) overlapped fractions. Reprinted from Ref. 11 with permission of Elsevier.

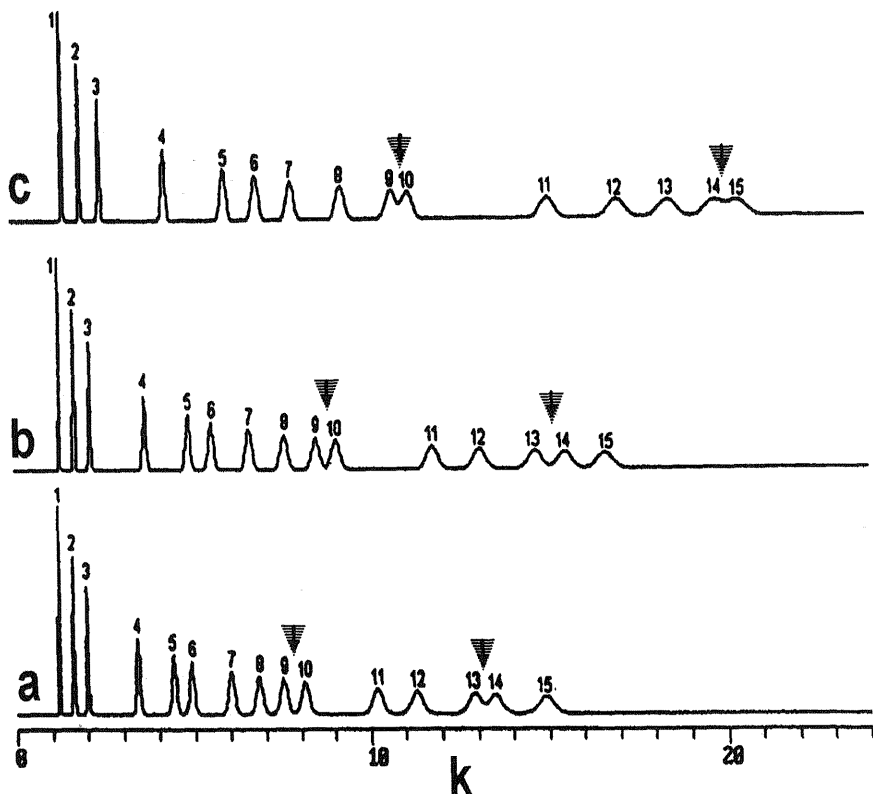


Figure 8.17 Chromatograms of a mixture of phenols in mobile phases containing 10% 2-propanol and diverse CTAB molar concentrations: (a) 0.12, (b) 0.10 and (c) 0.08. Peaks: (1) 4-benzamidephenol, (2) 4-hydroxybenzyl alcohol, (3) 4-hydroxyphenemethyl alcohol, (4) 4-hydroxybenzyl cyanide, (5) 4-hydroxyacetophenone, (6) 4-hydroxybenzaldehyde, (7) phenol, (8) 4-fluorophenol, (9) 4-hydroxypropiphenone, (10) 4-methylphenol, (11) 4-nitrophenol, (12) 4-hydroxybenzophenone, (13) 4-isopropylphenol, (14) 4-hydroxydiphenylmethane, and (15) 4-tert.-butylphenol. Reprinted from Ref. 11 with permission of Elsevier.

criteria; the global resolution for the valley-to-peak and overlapped fractions should both be high.

Other additional factors should be considered in the selection of a mobile phase. Thus, an optimum found in a region of large variation in the

global resolution function will not be adequate in practice, as the errors in the prediction of the retention and in the preparation of the mobile phases may lead to results different from those expected. For complex response surfaces showing several maxima and minima, additional experimental mobile phases should be prepared in the region where the optimum appears. In other cases, the optimum will correspond to a high experiment duration, and mobile phases giving shorter retention times will be more acceptable.

VIII.5. Selected Examples

Using the above strategy, the profile of a chromatogram can be predicted on the basis of a limited number of experiments, even though these experiments were relatively far apart in the variable space. An example of this prediction capability is the separation of a mixture of two diuretics (chlorthalidone and amiloride) and five steroids (boldenone, testosterone, methyltestosterone, medroxyprogesterone acetate and dydrogesterone) with mobile phases of SDS and acetonitrile. The six experimental mobile phases used for the prediction were 0.075 M SDS, 0.20 M SDS, 0.075 M SDS-10% acetonitrile, 0.14 M SDS-10% acetonitrile, 0.075 M SDS-20% acetonitrile, and 0.20 M SDS-20% acetonitrile [17]. The position of the maximum of the peaks was calculated using eq. 8.37, and the efficiencies and asymmetry factors were interpolated from the values obtained with the three experimental mobile phases closer to the predicted mobile phase from those available. The overlapped fractions were used to optimize the resolution. Fig. 8.18 shows the chromatograms of the mixture of diuretics and steroids, eluted with mobile phases of 0.14 M SDS (Fig. 8.18a) and 0.18 M SDS-17% acetonitrile (Fig. 8.18b). As observed, predicted and experimental chromatograms, located in regions far away from each other in the variable space, are in good agreement.

Another example of the good performance of the models shown in this chapter is given in Fig. 8.19, which shows the predicted and experimental chromatogram for a mixture of four diuretics, three β -blockers and a vasodilator, eluted with a mobile phase of 0.15 M SDS and 7% 1-propanol at pH 3 [14].

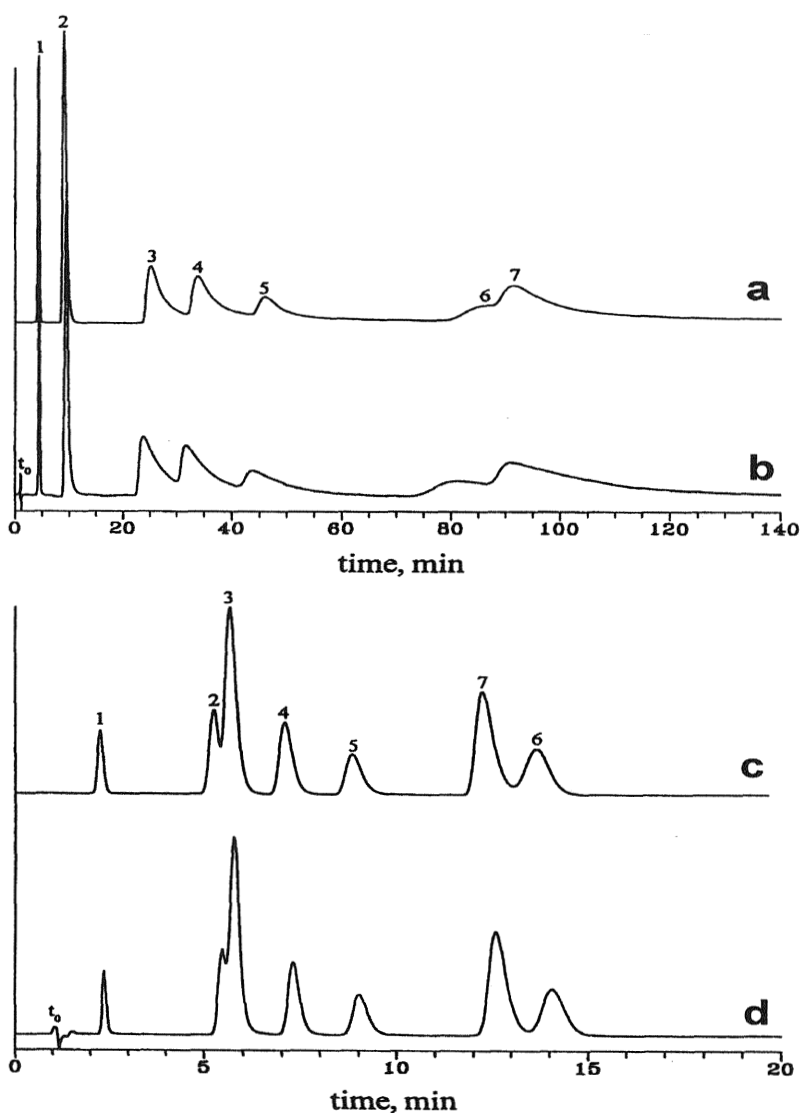


Figure 8.18 Predicted (a,c) and experimental (b,d) chromatograms for the separation of a mixture of diuretics and steroids, eluted with mobile phases of 0.14 M SDS (a,b) and 0.18 M-17% acetonitrile (c,d). The prediction of the position of the peaks was made with eq. 8.37, and the shape of each peak with eqs. 8.49-8.53. The area of the peaks was not normalized. Compounds: (1) chlorthalidone, (2) amiloride, (3) boldenone, (4) testosterone, (5) methyltestosterone, (6) medroxyprogesterone acetate, and (7) dydrogesterone. Reprinted from Ref. 27 with permission of the American Chemical Society.

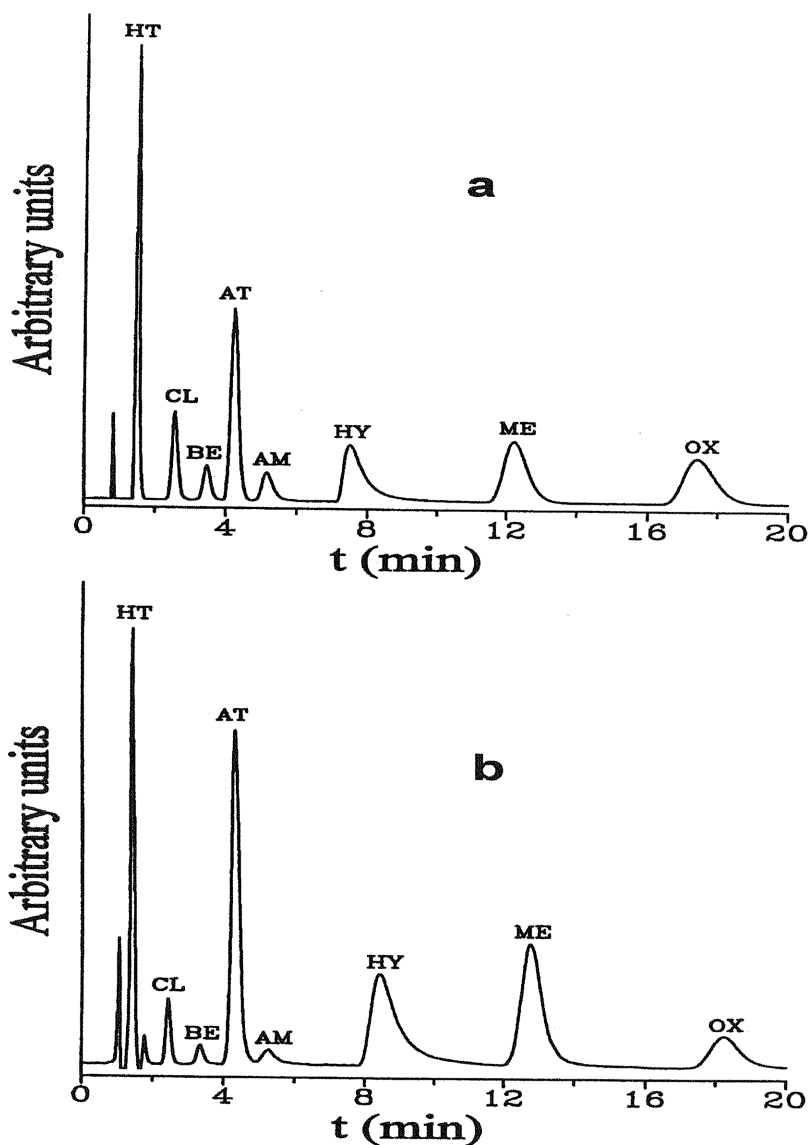


Figure 8.19 Predicted (a) and experimental (b) chromatograms of a mixture of compounds eluted with a 0.15 M SDS-7% 1-propanol mobile phase, buffered at pH 3.0. Compounds: hydrochlorothiazide (HT), chlorthalidone (CL), bendroflumethiazide (BE), atenolol (AT), amiloride (AM), hydralazine (HY), metoprolol (ME), and oxprenolol (OX). Reprinted from Ref. 14 with permission of the Royal Society of Chemistry.

IX. EASY MODELING AND OPTIMIZATION IN MLC WITH THE MICHROM SOFTWARE

The use of the optimization procedure described in this chapter can be assisted by the *MICHROM* software. Torres Lapasió was interested by the general treatment of chromatographic data using personal computers [9-11, 29]. He wrote a suite of programs which he called *MICHROM* (from Micellar Chromatography). The *MICHROM* software includes the modeling of the retention behavior and shape properties of chromatographic peaks, and the optimization of the resolution of mixtures.

The first release of *MICHROM* was developed to take part in all the stages of the analytical process [29]. It allows the determination of dead times, smoothing of chromatograms, measurement of peak parameters, modeling of skewed peaks and deconvolution of overlapped peaks. It also includes several tools for the experimental design, optimization of mobile phase composition (concentrations of surfactant and modifier at a fixed pH), management of sets of data, optimization and regression analysis, and simulation of chromatograms.

The user can interact with the software at several levels, from a semiautomatic to a fully manual mode. In the manual mode, the progressive changes with mobile phase composition of the predicted chromatograms can be graphically observed. Routines for the graphical representation of chromatograms, resolution surfaces and contour maps are implemented to better display the available information. All this environment allows a fast, reliable and easy resolution of chromatographic problems of diverse nature and complexity. *MICHROM* has been used in our laboratories since 1994. Several analytical procedures, commented in Chapters 10 to 12, were developed with the aid of this software.

This book is accompanied with a beta version of the second release of *MICHROM*, which contains only two modules dedicated to the modeling of the retention and further optimization of mobile phase composition. *MICHROM* (release 2.0) includes important improvements with respect to the first release. Problems involving three factors (concentrations of surfactant and modifier, and pH) are considered. Advances in modeling and

optimization are implemented, and the graphical interface is more flexible. The use of *MICHRUM* will reveal the great power of MLC in the prediction of chromatographic data. It is strongly recommended to read Appendix I before using the software.

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9

Quantitation of Hydrophobicity

I. INTRODUCTION

I.1. Logarithm of Octanol-Water Partition Coefficient as a Measure of Hydrophobicity

Hydrophobicity is commonly understood as a measure of the relative tendency of a solute to prefer a nonaqueous rather than an aqueous environment, or as a measure of the tendency of two (or more) solute molecules to aggregate in aqueous solution. The hydrophobicity of solutes is a relative property and depends mostly on the environment. When comparing the behavior of various solutes in the same environment, a quantitative scale can be established to demonstrate the abilities of individual solutes to participate in hydrophobic interactions.

The partition coefficient in the biphasic solvent system 1-octanol-water, P_{ow} , was proposed as a measure of hydrophobicity of organic compounds in the early 1960s by Hansch [1]. Since that time it has become the standard for measuring hydrophobicity. Large compilations exist of $\log P_{ow}$ data. The $\log P_{ow}$ hydrophobicity scale has the advantage of its universality, continuity and additive nature. On the basis of this additive rule, in order to estimate the partition coefficient values for new compounds, Hansch and Leo [2] derived constants for different functional groups.

However, despite numerous efforts made by many researchers using a variety of techniques, the measurement of P_{ow} is still problematic. The conventional shake-flask method has several disadvantages such as being tedious and time-consuming. Also, the solute must be inherently pure and available in reasonable quantities. Values of $\log P_{ow}$ in excess of about 6 are very difficult to measure, due to the very low concentration of solute in the aqueous phase.

Many attempts have been made to determine P_{ow} by other means different to the classical shake-flask method, which include countercurrent chromatography [3], and Reversed-Phase Liquid Chromatography (RPLC) with C8, C18, 1-octanol coated C18 and phenyl stationary phases [4]. The establishment of a correlation between retention data in RPLC and $\log P_{ow}$ assumes that the extent of chromatographic retention reflects the hydrophobicity of a solute. This approach is known as quantitative structure-retention relationships (QSRR) [5]. Chromatographic techniques offer a number of advantages over the static method. A great amount of relatively precise and reproducible data can be readily obtained, and the determinations are rapid and easy to be automated. Only a very small amount of sample is required, a wide dynamic range may exist and the impurities present in the sample can be simultaneously separated.

All conditions can be kept constant in a chromatographic process. Solute structure becomes thus the single most independent variable in the system. However, each partitioning chromatographic system yields an individual scale of hydrophobicity. The question arises whether different chromatographic hydrophobicity parameters should be used for predictive purposes or whether a chromatographic system should be developed that mimics the $\log P_{ow}$ hydrophobicity scale. In either case, the chromatographic measures of hydrophobicity should be defined and reproducible.

Micellar Liquid Chromatography (MLC) has shown to be an interesting RPLC technique to measure the hydrophobicity of solutes. Diverse contributions of several authors in the fields of the relationships between the retention with micellar eluents and the number of carbon atoms in homologous series, several descriptors of hydrophobicity, such as $\log P_{ow}$, and the biological activity of compounds, are reviewed in this chapter.

1.2. Solute Interactions in Micellar Eluents

Micelles are dynamic structures where a rapid exchange of surfactant monomers takes place with bulk solution, with other micelles, and with surfactant molecules adsorbed on any solid surface. Compounds solubilized by these systems participate in similar equilibria; they exist in dynamic equilibrium with bulk solvent, micellar pseudo-phase, and any surface

present. In MLC, the solutes partition from bulk solvent into micelles and into the stationary phase.

Khaledi et al. [6, 7] were concerned with the similarities and differences in retention behavior between the mode of RPLC which employs micellar eluents and that with aqueous-organic solvents. These techniques share the basic components of an RPLC system, that is, a nonpolar stationary phase and a polar aqueous mobile phase. The hydrophobicity of solutes should play an important role in governing the retention in both systems, which is easily controlled by adjusting solute-mobile phase interactions. However, the differences in interaction mechanism can cause significant differences in retention behavior.

Aqueous-organic solvents are homogenous, while micellar media are microscopically nonhomogeneous. Micelles are amphiphilic aggregates with anisotropic microenvironments that provide both hydrophobic and electrostatic sites of interaction with solutes. Three sites of solubilization can be identified in the micelles: the core (hydrophobic), the surface (hydrophilic) and the palisade layer (the region between the head group and the core). Solutes are solubilized in the micellar phase depending on the nature of solutes and micelles. Hydrophobic neutral solutes enter into the core of the micelle, relatively polar solutes are inserted in the palisade between surfactant molecules, and highly polar solutes can remain outside the micelle, adsorbed on its surface through electrostatic interactions. Ionic solutes will be attracted to the surfactant ionic groups in micelles, or repelled, depending on their charge. The interactions of solutes with micelles can occur through three different mechanisms: pseudo-phase extraction (partitioning), solute-surfactant coassembly (micellization), and surface adsorption. These interactions create a unique situation in which solutes occupying various locations in/on micelle may experience different microenvironment polarities in a given micellar mobile phase.

The characteristics of alkyl-bonded stationary phases in RPLC with micellar and aqueous-organic mobile phases are another noteworthy difference. The extraction of organic solvent by the grafted alkyl phase depends on the composition of the aqueous-organic mobile phase, which has a profound effect on retention. With micellar eluents, however, alkyl-bonded phases may be modified with an approximately constant concentration of

monomers of surfactant which is approximately equal to the critical micellar concentration (cmc). As a result, the structure of the stationary phase are independent of mobile phase composition. In other words, solutes experience a stationary phase with unchanged characteristics at different compositions of the micellar mobile phase. On the other hand, the adsorption of surfactant monomers on the stationary phase reduces the silanophilic interactions and increases the hydrophobicity of the stationary phase.

II. RETENTION BEHAVIOR OF HOMOLOGOUS SERIES

II.1. Correlations between Retention Factors and Carbon Number

The chromatographic behavior of alkyl homologous series is useful for the investigation of both mobile and stationary phase contributions to the retention mechanism in RPLC, and for the calibration of retention in such systems. The regular linear increase of retention due to the addition of a methylene group is recognized as a measure of hydrophobic interaction in a given RPLC system. Also, the existence of such a linear relationship makes the retention study of a homologous series particularly attractive for comparative purposes. The chromatography of these compounds is quite interesting and provides valuable information about the differences between micellar and aqueous-organic mobile phases.

a) Log k vs. n_C or k vs. n_C Correlations

With aqueous-organic mobile phases, the logarithm of the retention factor, k , is linearly related to the number of carbon atoms or repeat units in the normal chain of the homologous series, n_C , in the following form:

$$\log k = \log \alpha(\text{CH}_2) n_C + \log \beta \quad (9.1)$$

although some deviations from linearity have been noted [8]. The slope, $\log \alpha(\text{CH}_2)$, is a measure of methylene or hydrophobic selectivity, defined as

the ratio of the retention factors of two solutes differing from one another by a methylene group:

$$\alpha(\text{CH}_2) = k_{n+1}/k_n \quad (9.2)$$

while the intercept, $\log \beta$, reflects the specific interactions between the functional group of the molecules, and mobile and stationary phases.

The presence of micelles in the aqueous mobile phase of RPLC has a profound effect on the chromatographic characteristics of homologous series. Khaledi et al. [6, 7] and Borgerding et al. [9] reported that, in these separations, it is usually k (and not $\log k$) which is linearly related to the number of carbons:

$$k = a n_c + b \quad (9.3)$$

where a and b are fitting coefficients without any physical meaning. The plot of $\log k$ vs. n_c for these systems has a clear curvature, which indicates that $\log \alpha(\text{CH}_2)$ changes with the number of carbons of the homologous compounds. A quadratic equation would provide a better correlation:

$$\log k = (\log \gamma) n_c^2 + (\log \alpha) n_c + \log \beta \quad (9.4)$$

The relationship between $\log k$ and n_c for aqueous-organic RPLC has also been explained through a quadratic equation [8], however frequently, the coefficient of the second degree term is small enough to neglect this term; a linear correlation is thus observed. Apparently, this is not the case for the micellar and hybrid mobile phases (Fig. 9.1).

An estimation of how well the data points fit a straight-line is often made by examination of the correlation coefficient (r or r^2); this statistic is however easily misinterpreted because nonlinear data in character may give

a relatively high r^2 value. In order to confirm how the regression explains the variability of the data, the Fisher coefficient (F) can be used. Large F values ensure good agreement between data and the linear models.

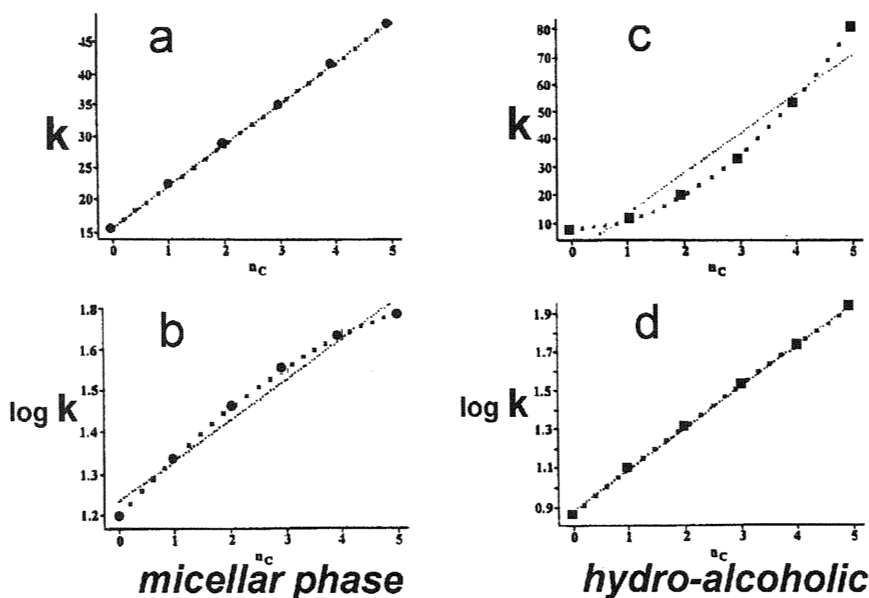


Figure 9.1 Linear and quadratic fits for k and $\log k$ vs. number of carbon atoms for n -alkylbenzenes. Symbols: experimental points ($\blacksquare$), second degree polynomial regression points ($\times$), linear regression points ($\cdots$). Mobile phases: 0.2 M SDS (a, b), and 2-propanol-water 35:65 (v/v) (c, d). Reprinted from Ref. 6 with permission of the American Chemical Society.

A linear behavior between k and n_c was found for n -alkylbenzenes eluted with pure micellar eluents of the anionic sodium dodecyl sulfate (SDS), cationic cetyltrimethylammonium bromide (CTAB), and nonionic poly[oxyethylene(10 or 23)]dodecanol (Brij® 22 or Brij® 35), and with hybrid eluents containing SDS or CTAB and 2-propanol. The homologues of n -alkylphenones eluted with pure and hybrid mobile phases of CTAB showed the same behavior, but linear relationships were observed between $\log k$ and n_c for these compounds eluted with SDS mobile phases, the same type of correlation found in aqueous-organic systems. It seems that the

correlation between k and n_C depends on the homologous series and surfactant type.

Table 9.1 Methylene Selectivity between Successive Members of Homologous Series, Eluted with Micellar Mobile Phases in a C8 Column [7]

Alkylbenzenes	0.06 M SDS	0.10 M SDS	0.20 M SDS	0.07 M CTAB	0.15 M CTAB	0.18 M CTAB
<i>n</i> -Hexylbenzene ^a	-	-	-	1.14	1.11	1.11
<i>n</i> -Pentylbenzene	-	1.20	1.16	1.13	1.11	1.12
<i>n</i> -Butylbenzene	-	1.27	1.20	1.13	1.16	1.13
<i>n</i> -Propylbenzene	1.39	1.31	1.24	1.21	1.18	1.18
Ethylbenzene	1.47	1.36	1.26	1.26	1.19	1.20
Toluene ^b	1.76	1.55	1.38	1.53	1.29	1.23
Alkylphenones						
<i>n</i> -Hexaphenone	1.40	1.37	1.36	1.27	1.23	1.23
<i>n</i> -Valerophenone	1.46	1.41	1.39	1.31	1.27	1.26
<i>n</i> -Butyrophenone	1.56	1.49	1.43	1.41	1.32	1.31
<i>n</i> -Propiophenone	1.59	1.50	1.41	1.62	1.45	1.42

^a $\alpha(\text{CH}_2) = k_{\text{hexylbenzene}}/k_{\text{pentylbenzene}}$.

^b $\alpha(\text{CH}_3) = k_{\text{toluene}}/k_{\text{benzene}}$.

It should be noted that the nonlogarithmic behavior (eq. 9.3) of alkylbenzenes and alkylphenones did not change upon addition of up to 20% of 2-propanol [7]. This was interpreted as follows: The addition of 2-propanol reduces solute partitioning into micelles. The stationary phase resembles more an aqueous-organic (water-propanol) system since the adsorbed surfactant layer is reduced by propanol additions (see Chapter 4).

However, that are still the solute-micelle interactions which play a major role in controlling the retention behavior in propanol-surfactant systems.

Table 9.2 Methylene Selectivity between Successive Members of Homologous Series, Eluted with Aqueous-Organic Mobile Phases in a C8 Column [7]^a

Alkylbenzenes	50% ACN	70% ACN	60% MeOH	90% MeOH	35% 2-PrOH	50% 2-PrOH
<i>n</i> -Pentylbenzene ^b	1.56	1.34	1.77	1.16	1.55	1.30
<i>n</i> -Butylbenzene	1.53	1.31	1.75	1.19	1.58	1.29
<i>n</i> -Propylbenzene	1.55	1.30	1.72	1.13	1.66	1.31
Ethylbenzene	1.48	1.27	1.61	1.12	1.61	1.28
Toluene ^c	1.47	1.25	1.62	1.13	1.67	1.31
Alkylphenones						
<i>n</i> -Hexaphenone	1.54	1.31	1.73	1.13	1.68	1.33
<i>n</i> -Valerophenone	1.51	1.27	1.69	1.13	1.67	1.31
<i>n</i> -Butyrophenone	1.50	1.26	1.59	1.08	1.65	1.32
<i>n</i> -Propiophenone	1.56	1.22	1.56	1.12	1.68	1.30

^a ACN = acetonitrile; MeOH = methanol; PrOH = propanol; v/v concentrations are given.

^b $\alpha(\text{CH}_2) = k_{\text{pentylbenzene}} / k_{\text{butylbenzene}}$.

^c $\alpha(\text{CH}_3) = k_{\text{toluene}} / k_{\text{benzene}}$.

The results commented above show that, in MLC, usually methylene selectivity decreases as the carbon number increases (Table 9.1). The methylene selectivity between *n*-pentyl- and *n*-butylbenzenes is, for instance, smaller than between ethylbenzene and toluene. The observed variations are quite small and in certain cases their statistical significance might be questionable. However, the decrease in $\alpha(\text{CH}_2)$ values with an increase in the molecular size is observed in almost all cases. For aqueous-organic

mobile phases, variations in $\alpha(\text{CH}_2)$ measured for different successive pairs are also observed, but these are rather random without a regular trend (Table 9.2).

The variations in selectivity along an homologous series implies that a larger number of compounds are eluted per unit time with micellar mobile phases, as compared to the traditional aqueous-organic mobile phases. It can be observed in Fig. 9.2, that between *ca.* 30 and 120 min, three peaks are eluted with a 75:25 methanol-water mobile phase, as compared to six or more with an aqueous 6% Brij® 35 micellar mobile phase, although the ability to resolve solutes is less for the micellar phase. The observed selectivity difference is not attributable to a difference in solvent strength, as the alkylbenzenes begin to elute earlier with the methanol-water mobile phase.

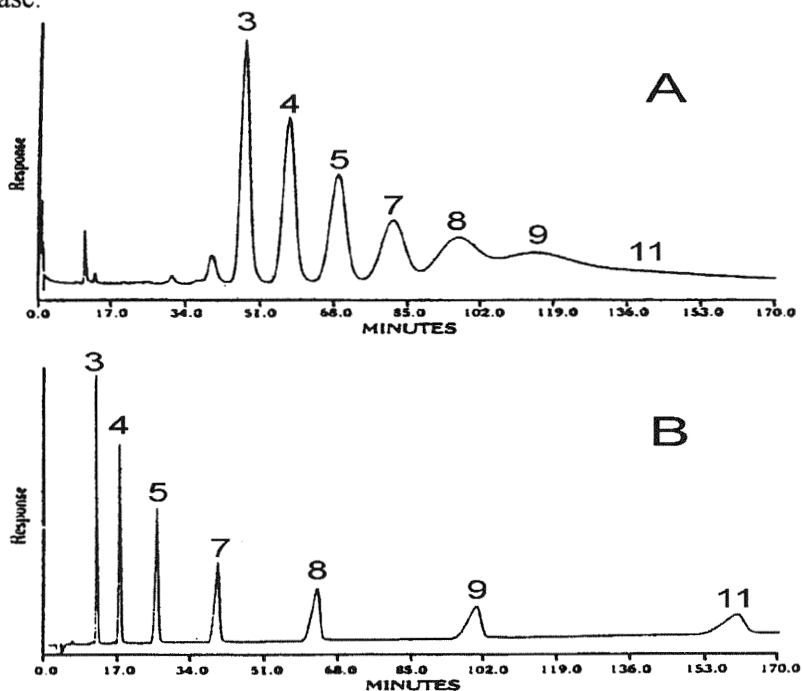


Figure 9.2 Chromatograms showing the separation of n-alkylbenzene homologues eluted with: (A) 6% Brij-35, and (B) methanol-water 75:25 (v/v). Elution order in A: propylbenzene, *ca.* 50 min, butylbenzene, amylbenzene, phenylheptane, phenyloctane, phenylnonane, and phenylundecane. Reprinted from Ref. 9 with permission of the American Chemical Society.

b) Effect of the Functional Group on Selectivity

Since $\alpha(\text{CH}_2)$ is the ratio of the retention factors of two compounds differing only in a methylene group, it should be independent of the series type for a given mobile and stationary phase system, such is the case for the aqueous-organic mobile phases. In contrast, as shown in Table 9.1, for micelles the $\alpha(\text{CH}_2)$ values are dependent on the type of series, as the methylene selectivity for alkylphenones are consistently greater than for alkylbenzenes [7].

The relative retention of homologous compounds in both alkylbenzene and alkylphenone series can be defined as:

$$\alpha(\text{CH}_2)_{n,Bz} = \frac{k(\text{C}_6\text{H}_5(\text{CH}_2)_n\text{CH}_3)}{k(\text{C}_6\text{H}_5\text{CH}_3)} \quad (9.5)$$

$$\alpha(\text{CH}_2)_{n,Ph} = \frac{k(\text{C}_6\text{H}_5\text{CO}(\text{CH}_2)_n\text{CH}_3)}{k(\text{C}_6\text{H}_5\text{COCH}_3)} \quad (9.6)$$

where toluene and acetophenone are used as the parent compounds. Therefore, the selectivity ratio of phenones/benzenes is:

$$\frac{\alpha(\text{CH}_2)_{n,Ph}}{\alpha(\text{CH}_2)_{n,Bz}} = \frac{k(\text{C}_6\text{H}_5\text{CO}(\text{CH}_2)_n\text{CH}_3)}{k(\text{C}_6\text{H}_5(\text{CH}_2)_n\text{CH}_3)} \frac{k(\text{C}_6\text{H}_5\text{CH}_3)}{k(\text{C}_6\text{H}_5\text{COCH}_3)} \quad (9.7)$$

or

$$\alpha(\text{CH}_2)_n \text{ ratio} = [\alpha(\text{CO})]_1 \times \frac{1}{[\alpha(\text{CO})]_2} \quad (9.8)$$

where $\alpha(\text{CO})$ is the selectivity of carbonyl group. For aqueous-organic solvents, $\alpha(\text{CH}_2)$ of two homologous pairs remains nearly constant at a different carbon number and the ratio in eq. 9.8 is unity. In micellar eluents, the changes in $\alpha(\text{CH}_2)$ with carbon number for alkylbenzenes are far smaller than those for alkylphenones. Therefore, the difference in $\alpha(\text{CH}_2)$ between both types of homologues increases with carbon number. The $\alpha(\text{CH}_2)$ ratio is different to unity, since the carbonyl selectivity between acetophenone and toluene is different from that of more hydrophobic compounds.

II.2. Why are the $\log k$ vs. n_c Plots Nonlinear?

The same authors that found the existence of a nonlinear relationship between k and n_c of homologous series in MLC, intended to give a rationalized explanation of this peculiar behavior. The different reported approaches are given below.

a) Microenvironment Polarities

The curvature in the $\log k$ vs. n_c plots, observed with micellar and hybrid mobile phases, was first attributed to the different locations (with different microenvironment polarities) in the micelle, for different members of a homologous series [6, 7]. Methylene selectivity decreases as the difference between mobile and stationary phase polarities is reduced. For a given mobile phase composition, the larger and more hydrophobic homologous compounds are located in a less polar environment of micelles, it is then conceivable to assume that these compounds experience a smaller change in their microenvironment polarity upon being transferred from the micellar pseudo-phase to the bonded alkyl stationary phase. The $\alpha(\text{CH}_2)$ value between *n*-pentylbenzene and *n*-butylbenzene is smaller than it is between ethylbenzene and toluene (Table 9.1), because the former pair is located in a more nonpolar environment than the latter.

For micelles, the typical selectivities for alkylphenones and alkylbenzenes are between 1.1 and 1.6 for SDS concentrations in the 0.06-0.5 M range, which is similar to the values observed for organic-rich eluents in aqueous-organic systems. The overall smaller $\alpha(\text{CH}_2)$ in MLC is an indication of how closely the environment of a methylene group in the micellar mobile phase resembles that of the alkyl-bonded stationary phase.

Micelles and grafted alkyl-bonded phases have a similar molecular organization, with chain molecules and a large surface/volume ratio, and their properties vary with depth from the surface. A constant amount of free surfactant adsorbed on the alkyl-bonded stationary phase makes the environments in the two systems even more similar. The appearance of the stationary phase is "micelle-like." It is not surprising that a methylene group does not find much difference between its environment in surfactant aggregates and in the alkyl-bonded phase. This situation is similar to that found in organic-rich conventional RPLC where the enrichment of an alkyl-bonded phase by organic solvent makes the stationary phase environment more similar to that of the mobile phase.

The effect of a polar functional group in a homologous series on the values of $\alpha(\text{CH}_2)$ can be also examined from the perspective of its contribution to the overall polarity of the nonionic molecules [7]. The carbonyl group of alkylphenones is located in a more polar medium of micelles than alkylbenzenes. As a result, the methylene groups of alkylphenones would see a different mobile phase environment and undergo a larger change in polarity as transferred from micellar eluent to stationary phase. A situation that does not exist in aqueous-organic solvents.

b) Free Energy of Cavity Formation

The linear relationship between k and n_c observed with micellar mobile phases may also result from the energetics unique to the pseudo-phase environment. In conventional RPLC with aqueous-organic mobile phases, the linear dependence between $\log k$ and n_c has been attributed to the direct proportionality between $\log k$ and the free energy of retention, which is in turn a linear combination of the free energy increments associated with the constituent parts of the molecule [8]. The predominant contribution to the free energy derives from the cavity formation within the mobile phase solvent structure that is required to accommodate the solute molecule transferred from the stationary phase:

$$\log k = -\frac{2.303 \Delta G_c}{RT} + \log c \quad (9.9)$$

This equation implies a constant contribution to the energy of transfer of the solute, between mobile and stationary phases, with each CH_2 increment in the chain length of the homologue:

$$\Delta\Delta G^\circ(\text{CH}_2) = -RT \ln \alpha(\text{CH}_2) \quad (9.10)$$

In other words, since the free energy of cavity formation, ΔG_c , is a function of the cavity surface area, it is proportional to the regular increase in molecular volume upon addition of each homologous structural unit.

In contrast, for MLC, it has been postulated that the free energy of cavity formation would not contribute to the overall free energy of retention, because the cavity created when the solute partitions from the micellar pseudo-phase to bulk water would be lost when the solute partitions from bulk water to the surfactant-modified stationary phase, producing no net free energy change [9].

The retention of solutes in MLC can be described by the following equation:

$$\frac{1}{k} = \frac{K_{AM}}{\phi P_{WS}} [M] + \frac{1}{\phi P_{WS}} \quad (9.11)$$

where K_{AM} is the solute-micelle binding constant, ϕ is the phase ratio, P_{WS} the partition coefficient between stationary phase and water, and $[M]$ the concentration of surfactant in the form of micelles [10]. As was derived in Chapter 7 for a pair of compounds, the chromatographic selectivity is simply the difference between micelle and stationary phase selectivities:

$$\log \alpha(\text{CH}_2) = \log \alpha(\text{CH}_2)_{WS} - \log \alpha(\text{CH}_2)_{AM} \quad (9.12)$$

Equation 9.12 indicates that the net free energy of transfer of a methylene group from mobile phase to stationary phase is the difference between the

free energy of transfer from bulk solvent (*e.g.*, water) to stationary phase, and from bulk solvent to micelle [7].

By analogy to the RPLC theory, the individual partitioning processes within the MLC mechanism would be expected to exhibit a logarithmic relationship for any partitioning process involving water as a phase [6, 9]:

$$\log K_{AM}(P_{WS}) = a n_C + b \quad (9.13)$$

The slope of this equation would be a measure of the free energy of transfer of a methylene group from bulk solvent to micelle (for $\log K_{AM}$ vs. n_C), or from bulk solvent to stationary phase (for $\log P_{WS}$ vs. n_C). The intercept represents again the interaction between the residue of the homologue with micelles or the stationary phase. However, the linear relationship cannot be extended to the higher homologues because negative intercepts are consistently encountered in the $1/k$ vs. $[M]$ regression analysis.

c) Solubility Limit Theory

The solubility limit model (see Chapter 5) appears to provide the best prediction of the retention for the entire homologous series [9, 11]. The behavior displayed by the homologous compounds can be qualitatively explained by focusing on the two independent equilibria between bulk water and stationary phase, and micelle and stationary phase. At low homologue number, the solutes are the most water soluble, so the retention is best described as being due to its distribution between the surfactant-modified stationary phase and the nonmicellar portion of the mobile phase, which is largely water. This equilibrium is typified by ordinary RPLC, and one expects a significant free energy of transfer for the methylene group. The slope of the $\log k$ vs. n_C curve reaches its largest value (Fig. 9.3). As the homologues become larger, they are less water soluble. In the limit of water insolubility, the homologues partition directly between the chemically similar micelles and hemimicellar modified stationary phase, the partition coefficient approaches unity and $\log k$ becomes independent of n_C . The $\log k$ vs. n_C curve flattens. For this process, ΔG_c must be nearly zero. Because ΔG_c is a function of homologue size, plots of $\log k$ vs. n_C are nonlinear.

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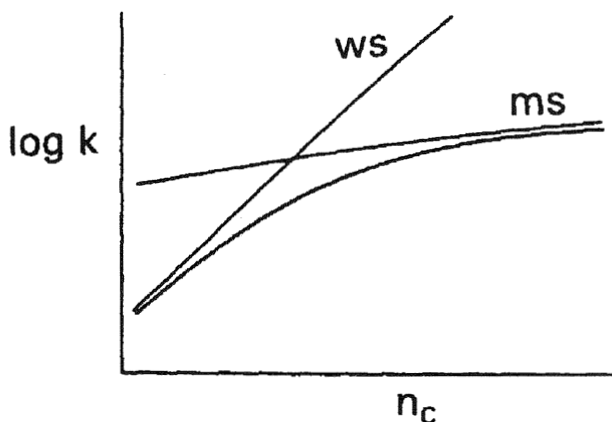


Figure 9.3 Idealized plot of eq. 9.17.

From the definition of k in MLC:

$$k = \frac{n_s}{n_w + n_m} \quad (9.14)$$

or the reverse:

$$\frac{1}{k} = \frac{n_w}{n_s} + \frac{n_m}{n_s} \quad (9.15)$$

n_s , n_w and n_m being the moles of solute in the stationary phase, bulk water and micelles, respectively. By substitution of the partition coefficients between mobile and stationary phases, P_{WS} and P_{MS} , in eq. 9.15:

$$\frac{1}{k} = \frac{V_w}{V_s P_{WS}} + \frac{V_m}{V_s P_{MS}} \quad (9.16)$$

Eq. 9.16 rewritten in terms of free energies yields the following:

$$\frac{1}{k'} = \phi_w \exp \left[\frac{\Delta G_{WS}^{fg} + n_C \Delta G_{WS}^c}{RT} \right] + \phi_m \exp \left[\frac{\Delta G_{MS}^{fg} + n_C \Delta G_{MS}^c}{RT} \right] \quad (9.17)$$

where the superscripts "fg" and "c" correspond to functional group and methylene, respectively. It is evident from eq. 9.17 that a linear relationship between $\log k$ and n_C is in principle not expected, nor a linear relationship between k and n_C .

The point on the curve in Fig. 9.3, where the break occurs, is the value of n_C that makes the WS part equal to the MS part in eq. 9.17, that is:

$$n_C = \frac{RT \ln \frac{\phi_w}{\phi_m} + (\Delta G_{WS}^{fg} - \Delta G_{MS}^{fg})}{\Delta G_{MS}^c - \Delta G_{WS}^c} \quad (9.18)$$

III. QUANTITATIVE STRUCTURE - RETENTION RELATIONSHIPS

In QSRR, two kinds of inputs are needed: a set of quantitatively comparable retention data for a sufficiently large number of solutes (dependent variable) and various quantities assumed to reflect structural features of the solutes

(independent variables). Through the use of diverse chemometric techniques, the retention parameters are characterized in terms of various combinations of solute descriptors or in terms of systematic knowledge extracted (learned) from these descriptors. Good QSRR can provide otherwise inaccessible information of the solutes and the chromatographic system involved: (i) the prediction of the retention for a new solute, (ii) the most informative structural descriptors, (iii) the molecular mechanism of separation operating a given chromatographic system, and (iv) complex physicochemical properties. The basic concepts on QSRR explained in this chapter have been extracted mainly from reviews written by Kaliszan [5, 12] and Dorsey and Khaledi [4], and should be consulted to extend the knowledge on this field.

III.1. QSRR in Conventional RPLC

a) The Logarithmic Correlation

Retention in RPLC with aqueous-organic mobile phases depends mainly on hydrophobic interactions. In general, some correlation between RPLC retention data and $\log P_{ow}$ is observed for a given series of solutes. The observed relationships support the assumption that in RPLC, partitioning rather than adsorption processes are decisive for retention.

In RPLC, the relationship between retention and $\log P_{ow}$ is often expressed in the logarithmic form as:

$$\log k = a \log P_{ow} + b \quad (9.19)$$

which is a special case of the Collander equation that predicts linear relationships between the logarithm of the partition coefficients measured in two different partitioning systems, provided that solute-solvent interactions are similar in the two systems [1]:

$$\log P_2 = a \log P_1 + b \quad (9.20)$$

One disadvantage of the RPLC system is that it offers distinct individual hydrophobicity measurements. For example, at a fixed concentration of a given solvent, solute X may appear more hydrophobic than solute Y, whereas the reverse seems to be true at another concentration. The presence of organic modifier in mobile phase makes the interactions determining chromatographic retention extremely complex. High concentrations of organic modifier weaken the hydrophobic effect, and consequently, result in under or overestimation of hydrophobicity. Therefore, an initial question that must be addressed in these studies is both the choice of organic modifier and its concentration.

It has been generally recognized that the best measure of hydrophobicity is the retention factor with a mobile phase of 100% water, k_w . The advantages of using k_w are that it is independent of any specific organic modifier effects, it reflects polar-nonpolar partitioning in a manner similar to shake-flask measurements, and is dependent on solute's structure and polar functionalities. However, k_w is difficult to obtain experimentally for most compounds because of prohibitively long retention times, and usually it must be obtained by the extrapolated intercept of a plot of $\log k$ vs. volume percent organic solvent. While these plots are linear for a narrow range of organic solvent, it is well known that deviations from linearity occurs if a wide range is examined. Even more troubling is that for a given solute-column pair, extrapolation of $\log k$ vs. volume percent organic solvent gives significantly different intercepts for different organic solvents.

b) Congenerity of Solutes

The correlations between RPLC retention factors and $\log P_{ow}$ are limited to congeneric compounds. This means that different relationships will probably be observed for different sets of compounds, because of the complex nature of the factors that contribute to retention, which is drastically different from the transfer of solutes between two isotropic aqueous and organic solvents. The term congeneric is not just limited to homologous compounds of a given series. Different solutes from various classes (hydrogen bond donors, hydrogen bond acceptors or nonhydrogen bond donors) can be classified as a congeneric group if they show similar partitioning behavior in solvent systems with dissimilar phase polarities. Accurate definition of the term congeneric is however difficult and is certainly open to different interpretations.

Several researchers have attempted to find a single equation that relates $\log k$ in RPLC to $\log P_{ow}$ for a wide range of compounds that differ in size, shape, functional groups and type of interactions. The primary approach has been to adjust the chromatographic conditions (i.e., composition of mobile and stationary phases) such that the transfer of solutes from the aqueous media to the nonpolar bonded phases of RPLC closely resembles the partitioning process in an octanol-water system.

Hydrophobicity is as much a phobia against the aqueous environment as a "philia" toward nonpolar species, and thus, the chemistry involved in the contact of a solute with the stationary phase cannot be neglected. In order to apply the Collander equation to the relationship between the retention and $\log P_{ow}$, a similar mechanism should exist between the solutes and the organic phases, in addition to a similar molecular interaction. However, octanol is an isotropic liquid while the alkyl-bonded stationary phases used in RPLC are anisotropic and heterogeneous media, whose properties vary with depth from the interface. Also, the structure and composition of the alkyl-bonded phase depend upon the type and concentration of organic cosolvent in the aqueous mobile phase. Furthermore, the residual silanol on the silica surface can influence the retention of certain solutes. The accessibility of silanol groups (which are reported to be solvated with water and/or hydrogen bonding organic cosolvent) depends on the composition of mobile phase.

For years, octadecyl-bonded silica (ODS) stationary phases have been commonly employed for hydrophobicity studies. However, the retention data obtained using individual ODS columns—even though they are basically the same type of material—are hardly comparable. The stationary phases vary in terms of endcapping, surface area of the bonded phase, carbon content and concentration of residual silanol groups. Another disadvantage of ODS reversed-phase materials is their instability at $\text{pH} > 8$. With these limitations in mind, researchers have attempted to introduce reversed-phase materials for the construction of a universal, continuous chromatographic hydrophobicity scale (*e.g.*, specially deactivated ODS-based phases and polymer-coated ODS). Better knowledge of the nature of the partitioning processes involved may make possible the design of systems, in which the chromatographic retention of a certain solute depends solely on its relative hydrophobicity.

III.2. QSRR in MLC

a) Correlations between Retention Factors and $\log P_{ow}$

Some reports have appeared in the literature showing MLC as a promising technique for the quantitation of hydrophobicity [3]. It appears that retention data obtained with micellar eluents in RPLC can be used in QSRR analysis. Micelles are unique media which offer flexibility in controlling specific interactions along with hydrophobic forces, by the careful selection of surfactant (chain length and head group) and solvent additive.

Several studies have been published on the correlations between the retention in MLC and $\log P_{ow}$. Gago et al. [13] and González et al. [14] reported good correlations between $\log k$ and $\log P_{ow}$ for 11 monosubstituted benzenes, and groups of 12 to 16 polycyclic aromatic hydrocarbons (PAHs) (Fig. 9.4), respectively, eluted with micellar eluents of SDS, CTAB and Brij® 35 from C18 columns. The same was observed by Lavine et al. [15] for 21 aromatic compounds with a mobile phase of SDS and 1-propanol.

In contrast, Khaledi and Breyer [16] reported excellent correlations for k (instead of $\log k$) vs. $\log P_{ow}$ for anionic (SDS) and cationic (tetradecyltrimethylammonium bromide, C₁₄TAB) surfactants on C8 and phenyl-bonded stationary phases, with compounds having various functional groups. The $\log k$ vs. $\log P_{ow}$ plots consistently showed a curvature. The two sets of 16 and 35 compounds used by these authors with C8 and phenyl columns, respectively, covered more than 4-orders of magnitude in hydrophobicity and represented a relatively broad range of molecular interactions, molecular shapes and sizes. In a sense, the sets consisted of several noncongeneric compounds.

Figure 9.5 illustrates plots of k and $\log k$ vs. $\log P_{ow}$, for micellar and aqueous-organic mobile phases and a set of 16 aromatic compounds. As shown in Fig. 9.5b, there is a clear curvature in the plot of $\log k$ vs. $\log P_{ow}$ for the micellar eluent (a quadratic fit provides a better regression than a linear fit). Also, the r data in Table 9.3 indicate that for the micellar mobile phases, k vs. $\log P_{ow}$ fits are better than $\log k$ vs. $\log P_{ow}$ fits. Therefore, the same phenomenon appears in the $\log k$ vs. n_c and $\log k$ vs. $\log P_{ow}$ plots, which can be explained by the solubility limit theory proposed by Borgerding et al. [9]. For compounds with low $\log P_{ow}$ values, the solutes are relatively

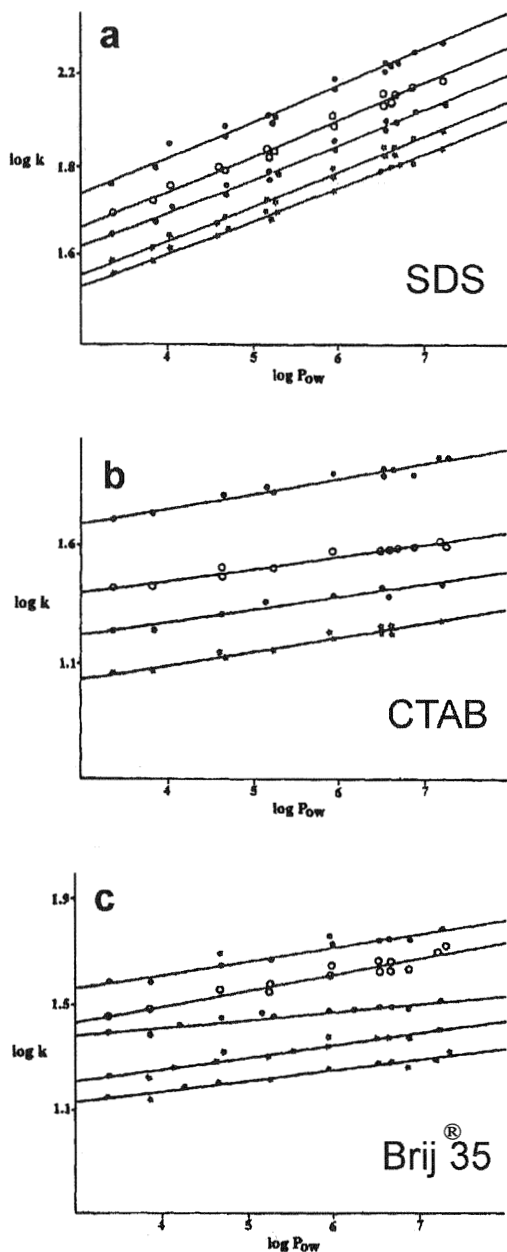


Figure 9.4 Plots of $\log k$ vs. $\log P_{ow}$ for several PAHs eluted with (surfactant molar concentration is given from top to bottom): (a) SDS (0.06, 0.08, 0.10, 0.13, 0.15 M); (b) CTAB (0.02, 0.04, 0.06, 0.08 M); (c) Brij-35 (0.02, 0.04, 0.06, 0.08, 0.10 M). PAHs: naphthalene, ace-naphthylene, fluorene, anthracene, phenanthrene, 9-methyl-anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, perylene, dibenz[ac]anthracene, dibenz[ah]anthracene, and benzo[ghi]perylene. Reprinted from Ref. 14 with permission of Vieweg Publishing.

Table 9.3 Comparison of the Correlation Coefficients of the Fittings k vs. $\log P_{ow}$ and $\log k$ vs. $\log P_{ow}$ for Micellar and Aqueous-Organic Eluents

Eluent	r	
	k vs. $\log P_{ow}$	$\log k$ vs. $\log P_{ow}$
<i>n</i> = 16 [16]		
0.04 M CTAB	0.970	0.925
0.04 M CTAB + 3% 2-propanol	0.980	0.933
0.08 M CTAB + 3% 2-propanol	0.965	0.910
0.12 M CTAB + 3% 2-propanol	0.959	0.913
0.04 M SDS + 3% 2-propanol	0.964	0.925
0.08 M SDS + 3% 2-propanol	0.969	0.930
0.12 M SDS + 3% 2-propanol	0.966	0.878
Methanol-water 70:30 (v/v)	0.873	0.901
Methanol-water 40:60 (v/v)	0.821	0.982
<i>n</i> = 11 [13]		
0.10 M SDS	0.969	0.988
0.016 M CTAB	0.946	0.997
0.05 M CTAB	0.984	0.984
0.10 M CTAB	0.989	0.987
0.016 M Brij.35	0.953	0.986
0.05 M Brij-35	0.980	0.972
Methanol-water	0.970	0.985

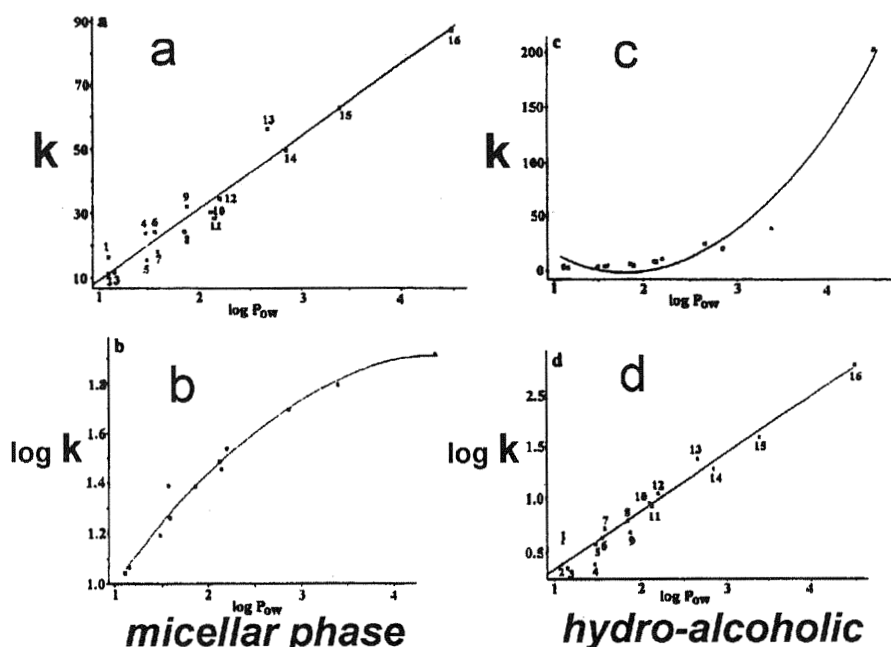


Figure 9.5 Plots showing the relationship between k and $\log k$ vs. $\log P_{ow}$ for 0.04 M C_{14} TAB-3% 2-propanol (a, b), and methanol-water 40:60 (v/v) (c, d). Compounds: (1) benzylamine, (2) benzyl alcohol, (3) acetanilide, (4) phenol, (5) benzaldehyde, (6) benzonitrile, (7) acetophenone, (8) nitrobenzene, (9) benzoic acid, (10) anisole, (11) benzene, (12) propiophenone, (13) butyrophenone, (14) chlorobenzene, (15) naphthalene, and (16) anthracene. Reprinted from Ref. 16 with permission of the American Chemical Society.

water soluble, so water-stationary phase partitioning plays its largest role. Highly hydrophobic solutes become insoluble in water, and the micelle-stationary phase equilibrium then becomes predominant. In this equilibrium, the two phases are chemically similar and the partition coefficient approaches unity, becoming independent of hydrophobicity. The $\log k$ - $\log P_{ow}$ curve flattens.

This explains why $\log k$ - $\log P_{ow}$ correlations improve when the most hydrophobic compounds are eliminated from the curve. Thus, for a 0.04 M CTAB-3% 2-propanol eluent on a C8 stationary phase, elimination of the more hydrophobic compounds from the cited set of 16 aromatic compounds (which reduced the upper limit of $\log P_{ow}$ from 4.45 to 2.19) resulted in a better regression coefficient for the $\log k$ vs. $\log P_{ow}$ fit as compared to k vs. $\log P_{ow}$. With the same mobile phase on a phenyl column, however, k vs. $\log P_{ow}$ provided better correlations for two sets of 28 and 23 compounds with different upper hydrophobicity limits of 4.88 and 2.69 [16].

The cationic micellar eluents of C_{14} TAB gave better linear correlations for k vs. $\log P_{ow}$ than the anionic surfactant SDS [16]. This might indicate that the cationic micellar systems have a similar hydrophilic/lipophilic balance to octanol-water, and/or the polar interactions between the solutes and the cationic head group better resemble those in octanol-water. For the chromatographic correlations, one should also note that the cationic surfactants adsorbed on the stationary phase can better shield the residual silanol groups on the silica surface, than SDS. Consequently, the reduction (or elimination) of the silanophilic interactions would increase the correlations between RPLC retention and $\log P_{ow}$.

Otherwise, the addition of alcohol to the micellar mobile phase seems to improve $\log k$ - $\log P_{ow}$ [17, 18] and k vs. $\log P_{ow}$ [16] linear correlations. The alcohol apparently provides an environment that is more closely related to that of octanol-water than pure aqueous micellar systems.

These results show that the type of general relationship depends not only upon the set of compounds but also on the characteristics of mobile and stationary phases. This was confirmed in a study of the chromatographic behavior of a group of 11 benzene derivatives and 12 PAHs, in SDS and CTAB micellar mobile phases modified with methanol, 1-propanol or 1-butanol at different percentages [19]. The authors demonstrated effectively that the hydrophobicity range of compounds is an important factor in the k - $\log P_{ow}$ or $\log k$ - $\log P_{ow}$ correlations. For the whole set of 23 aromatic compounds studied ($\log P_{ow}$ from 0.64 to 5.03), k always correlated better with $\log P_{ow}$ than $\log k$, irrespective of the nature of the surfactant present in the mobile phase, and the nature and percentage of the alcohol used as a modifier. However, for a group of 15 benzene and naphthalene derivatives

(log P_{ow} from 0.64 to 3.37), and for 10 monosubstituted benzenes (log P_{ow} from 0.64 to 2.84), k correlated better with log P_{ow} than log k only when CTAB was used, whereas with SDS log k -log P_{ow} correlations were similar to or even better than k -log P_{ow} . Thus, depending on the situation, either k or log k might better fit the data and one must therefore try both and use the one with the best correlation.

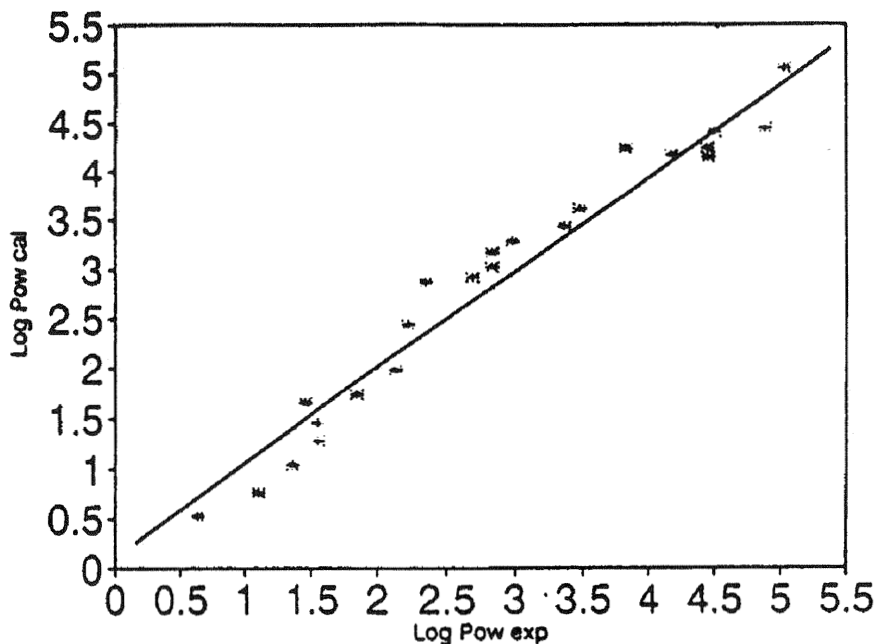


Figure 9.6 Calculated vs. experimental log P_{ow} values for 0.05 M CTAB-3% 1-propanol mobile phase for a group of benzene derivatives and PAHs. Reprinted from Ref. 19 with permission of Vieweg Publishing.

The values of log P_{ow} can be calculated from experimental k data, using the equation of the fitted straight-line. Figure 9.6 shows calculated log P_{ow} plotted vs. experimental values for a mobile phase of 0.05 M CTAB modified with 3% 1-propanol. The average of the relative error obtained between the calculated and experimental values was 9.6%. Finally, it should

be noted that deviations from the established correlations can be expected for cases where other factors (e.g., structural properties, other specific molecular interactions besides hydrophobicity) significantly contribute to the partitioning process.

b) Hydrophobicity of Amino Acids

Isoindoles of o-phthalaldehyde (OPA)/N-acetyl-L-cysteine (NAC) are commonly used for detection of amino acids ($R_1\text{CH}(\text{COOH})\text{NH}_2$) in RPLC. A particular hydrophobicity scale was established with the retention data of these derivatives in mobile phases of SDS at pH 3, using the glycine derivative as a reference [20]. Linear relationships were obtained between the ratios of $\log k$ of each amino acid derivative to $\log k$ of the glycine derivative (which was called quantitation of hydrophobicity index, QH), and $\log P_{\text{ow}}$ for the R_1 substituents (π_{R1}).

The interactions of the amino acid derivatives with a modified C18 column and micellar mobile phases of the anionic SDS are mainly of hydrophobic and electrostatic nature. The basic structure of the amino acid isoindoles is the same. Consequently, in the absence of electrostatic interactions, the hydrophobic character of the R_1 substituent should be responsible of the retention. The nitrogen atom in the isoindole heterocycle is nonprotonated at pH 3, and thus, electrostatic interactions can only exist with ionizable groups giving a positive charge to R_1 in the molecule. This occurs with arginine and histidine, which have amino groups that do not react with OPA.

Figure 9.7 shows $\log \text{QH}$ vs. π_{R1} plots for hybrid eluents of SDS and methanol, 1-propanol and 1-pentanol. For all these eluents, the correlation between $\log \text{QH}$ and π_{R1} was good. For the derivatives of alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, serine, and threonine, the QH index was less than unity and π_{R1} was negative or slightly positive. For these compounds, the order of hydrophobicity was the same according to the QH index and the π_{R1} value. Valine, leucine and isoleucine with $\text{QH} > 1$, also showed this behavior. On the other hand, for some compounds showing $\text{QH} > 1$, a differentiated behavior was observed. This was the case of the derivatives of arginine and histidine, which showed a

high QH index due to electrostatic attraction of the additional protonated amino group in the R_1 substituent with the anionic surfactant. Another separate group was formed by tyrosine, tryptophan and phenylalanine, because of the presence of an aromatic ring in the R_1 substituent.

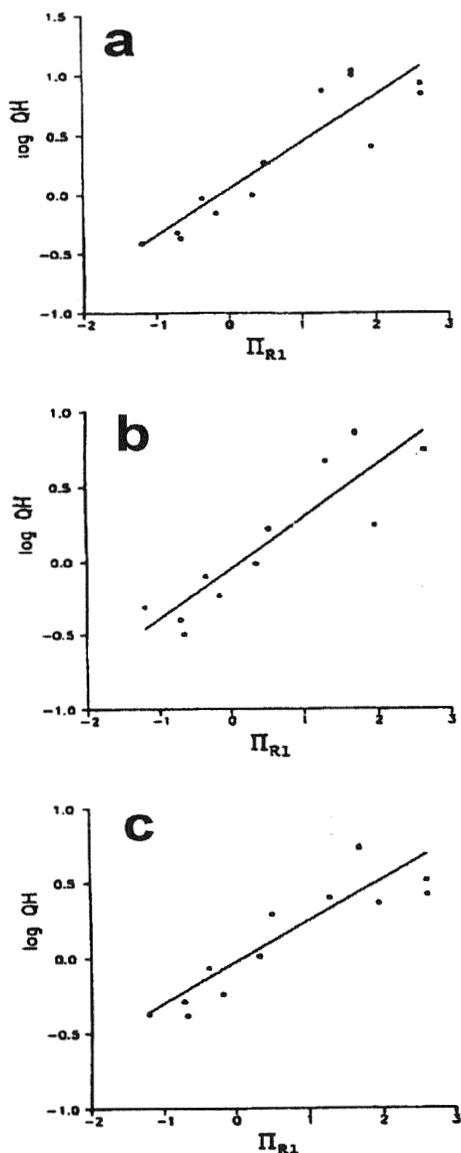


Figure 9.7 Plots of log QH index vs. log P_{ow} of R_1 substituent for amino acid OPA-NAC derivatives, eluted with 0.05 M SDS micellar mobile phase at pH 3 containing: (a) 5% methanol, (b) 3% 1-propanol, and (c) 1% 1-pentanol. Amino acids: alanine, asparagine, aspartic acid, glutamic acid, glutamine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine. Reprinted from Ref. 20 with permission of Vieweg Publishing.

c) An Apparent Linearity

In MLC, the retention is influenced by two competing equilibria of solute interactions with micelles in the mobile phase (controlled by K_{AM}) and their partitioning into the stationary phase (controlled by P_{WS}) (see eq. 9.11). Both partitioning processes depend on the hydrophobicity of the solute. The reciprocal of the intercept in eq. 9.11 is the retention factor at zero micelle concentration, k_o , which is a parameter similar to k_w , and as shown below, is useful in hydrophobicity measurements.

Let's now suppose that $\log k_o$ and $\log K_{AM}$ are correlated with the hydrophobicity of solutes:

$$\log k_o = A_o + A_1 \log P_{ow} \quad (9.21)$$

$$\log K_{AM} = B_o + B_1 \log P_{ow} \quad (9.22)$$

then eq. 9.11 becomes:

$$k = \frac{k_o}{1 + K_{AM} [M]} = \frac{10^{(A_o + A_1 \log P_{ow})}}{1 + [M] 10^{(B_o + B_1 \log P_{ow})}} \quad (9.23)$$

and in logarithmic form:

$$\log k = A_o + A_1 \log P_{ow} - \log(1 + [M] 10^{(B_o + B_1 \log P_{ow})}) \quad (9.24)$$

From eqs. 9.23 and 9.24, a nonlinear relationship should be expected between the retention of a compound (k or $\log k$) and $\log P_{ow}$, for a constant micellar concentration. Two extreme situations can be considered:

- (i) For solutes with low hydrophobicity (low $\log P_{ow}$) or for very low micellar concentrations in the mobile phase, the term $K_{AM} [M]$ is negligible and eq. 9.23 becomes:

$$k \approx k_0 = 10^{(A_0 + A_1 \log P_{ow})} \quad (9.25)$$

which gives a nonlinear relationship between k and $\log P_{ow}$. Besides, from eq. 9.24:

$$\log k \approx \log k_0 = A_0 + A_1 \log P_{ow} \quad (9.26)$$

which translates into a linear relationship between $\log k$ and $\log P_{ow}$.

- (ii) For highly hydrophobic solutes (high $\log P_{ow}$) or for high micellar concentrations in the mobile phase, eqs. 9.23 and 9.24 result in:

$$k = \frac{k_0}{K_{AM} [M]} = \frac{10^{(A_0 + A_1 \log P_{ow})}}{[M] 10^{(B_0 + B_1 \log P_{ow})}} \quad (9.27)$$

from where:

$$\log k = A_0 - B_0 + (A_1 - B_1) \log P_{ow} - \log [M] \quad (9.28)$$

Equation 9.27 provides an apparent linear relationship between k and $\log P_{ow}$, and eq. 9.28 describes a linear relationship between $\log k$ and $\log P_{ow}$.

Accordingly, when $\log k$ is plotted vs. $\log P_{ow}$, a break should occur when $K_{AM} [M] \approx 1$. The value of $\log P_{ow}$ for this point is:

$$\log P_{ow} = - \frac{\log [M] + B_o}{B_1} \quad (9.29)$$

As can be deduced from eq. 9.29, the linearity range of the plots increases when the micellar concentration in the mobile phase decreases. There is therefore an apparent parallelism with the fact that a decrease in organic modifier of aqueous-organic mobile phases, in conventional RPLC, results in better correlations of $\log k$ vs. $\log P_{ow}$.

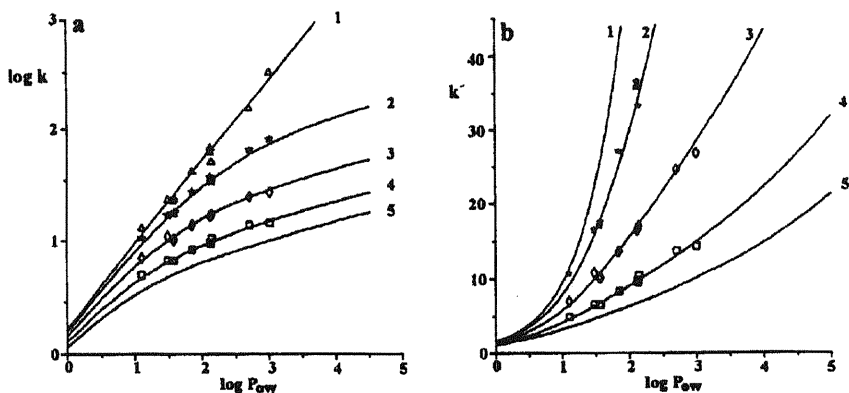


Figure 9.8 $\log k$ vs. $\log P_{ow}$ (a) and k vs. $\log P_{ow}$ (b) relationships predicted by eqs. 9.23 and 9.24 (solid lines), and experimental values (symbols) for a series of monosubstituted benzenes: acetanilide, acetophenone, benzaldehyde, benzene, benzonitrile, benzyl alcohol, benzylamine, bromobenzene, butyrophenone, hexaphenone, methyl benzoate, methyl phenyl ether, nitrobenzene, propiophenone, toluene, and valerophenone. Molar concentrations of SDS in mobile phase: (1, Δ) 0, (2, $\star$) 0.016, (3, $\diamond$) 0.05, (4, $\square$) 0.1, and (5) 0.15. Reprinted from Ref. 21 with permission of Elsevier.

Figure 9.8 depicts the modeled $\log k$ vs. $\log P_{ow}$ (left) and k vs. $\log P_{ow}$ (right) relationships for several neutral compounds, at various micellar concentrations, together with experimental points. In general, good agreement between predicted and experimental values was obtained. The

relationships between $\log k$ and $\log P_{ow}$ are nonlinear (curves 2-5), as predicted by eq. 9.24, although two different regions in each curve with an apparent linearity are observed at low and high $\log P_{ow}$ values. A decrease in micellar concentration expands the range of linearity. In contrast, as predicted by eq. 9.23, k - $\log P_{ow}$ relationships are always nonlinear.

Table 9.4 Statistical Analysis of Linear Regressions Between Chromatographic Parameters and $\log P_{ow}$ for Several Sets of Compounds [21].^a

Series	Stationary Phase/Surfactant	$\log P_{ow}$ range	n	Dependent Variable	r	F
I	C18/0.1 M SDS	2.13-4.60	9	$\log k$	0.931	45
				k	0.954	71
				$\log k_0$	0.989	308
				k_0	0.760	10
				$\log K_{AM}$	0.986	250
				K_{AM}	0.718	7
II	C18/0.1 M SDS	1.10-3.58	12	$\log k$	0.977	227
				k	0.961	133
				$\log k_0$	0.982	277
				k_0	0.913	55
				$\log K_{AM}$	0.856	28
				K_{AM}	0.922	57
III	C18/0.1 M CTAB	1.10-2.99	10	$\log k$	0.987	293
				k	0.991	430
				$\log k_0$	0.992	497
				k_0	0.868	25
				$\log K_{AM}$	0.979	182
				K_{AM}	0.866	25

Table 9.4 (continued)

Series	Stationary Phase/Surfactant	$\log P_{ow}$ range	n	Dependent Variable	r	F
IV	C18/0.05 M Brij® 35	1.10-2.99	10			
				$\log k$	0.959	91
				k	0.989	372
				$\log k_0$	0.993	608
				k_0	0.833	18
				$\log K_{AM}$	0.974	146
V	C8/0.12 M CTAB ^b	1.10-4.45	11	K_{AM}	0.817	16
				$\log k$	0.924	58
				k	0.966	139
				$\log k_0$	0.991	500
				k_0	0.863	29
				$\log K_{AM}$	0.987	354
VI	C18/0.12 M SDS ^b	1.10-4.45	11	K_{AM}	0.889	34
				$\log k$	0.951	95
				k	0.984	301
				$\log k_0$	0.991	534
				k_0	0.864	29
				$\log K_{AM}$	0.982	248
				K_{AM}	0.917	48

^a Series I: anthracene, benzene, biphenyl, 1-bromonaphthalene, 1-methylnaphthalene, naphthalene, pyrene, toluene and p-xylene. Series II-IV: acetanilide, acetophenone, benzaldehyde, benzene, benzonitrile, benzyl alcohol, benzylamine, bromobenzene, butyrophenone, hexaphenone, methyl benzoate, methyl phenyl ether, nitrobenzene, propiophenone, toluene and valerophenone. Series V-VI: acetophenone, anthracene, benzaldehyde, benzene, benzonitrile, benzyl alcohol, butyrophenone, chlorophenone, naphthalene, nitrobenzene and propiophenone.

^b Mobile phase with 3% 2-propanol.

d) *Log k_0 vs. log P_{ow} and log K_{AM} vs. log P_{ow} as a Means to Probe Solute Interactions in the Chromatographic System*

Log k_0 vs. log P_{ow} and log K_{AM} vs. log P_{ow} relationships have also been examined [16, 21, 22]. The results suggest that K_{AM} does not reflect the hydrophobicity of compounds as well as k_0 (Table 9.4). Figure 9.8 also reveals the superior capability of log k_0 data (curve 1 in Fig. 9.8a) with respect to log k or k to predict the hydrophobicity of solutes. Note also the greater sensitivity (slope) of the log k_0 -log P_{ow} plot.

Lavine et al. [15] made an interesting study, using a set of 21 aromatic compounds as probes, that demonstrates the interactions of solutes with mobile and stationary phases in MLC. In Fig. 9.9, log P_{ow} is plotted against log k for methanol-water and SDS mobile phases. It can be seen that the data set is divided into two groups. The first group of compounds (group A) is mainly composed of monosubstituted benzenes, whereas the second group (group B) consists entirely of phenols. Compound 3 (2,4-dinitrophenol), which does not belong to any group, existed mainly in the anionic

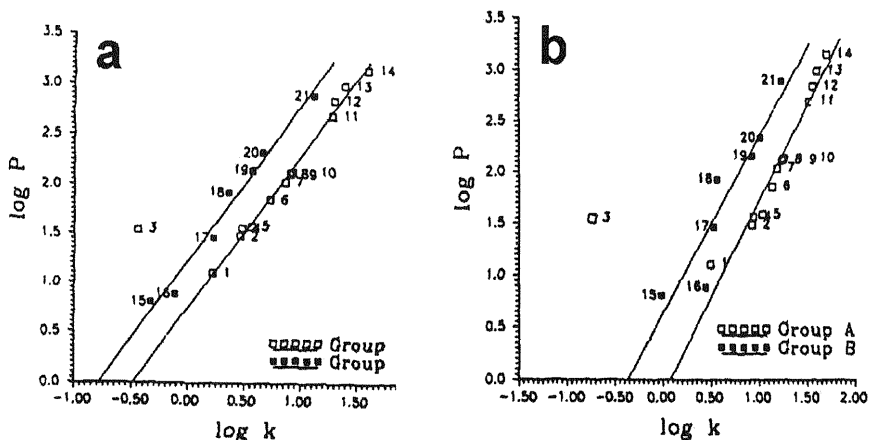


Figure 9.9 Plots of log P_{ow} vs. log k for a series of 21 aromatic compounds. A C18 column was used with (a) methanol-water 50:50 (v/v), and (b) 0.05 M SDS with 2% 1-propanol. Compounds: (1) benzyl alcohol, (2) benzaldehyde, (3) 2,4-dinitrophenol, (4) benzonitrile, (5) acetophenone, (6) nitrobenzene, (7) p-nitroanisole, (8) methylbenzoate, (9) anisole, (10) benzene, (11) toluene, (12) chlorobenzene, (13) bromobenzene, (14) ethylbenzene, (15) resorcinol, (16) catechol, (17) phenol, (18) p-nitrophenol, (19) o-chlorophenol, (20) o-bromophenol and (21) 2,4-dichlorophenol. Reprinted from Ref. 15 with permission of Elsevier.

form at the pH of the mobile phase (pH 6.3) and therefore, eluted off the column in the dead volume. The only difference between the two data sets (methanol-water and SDS mobile phases) is that catechol lies in group A instead of group B, but there is no difference in the elution order of the compounds. The slopes of the lines drawn through the sets of points are approximately equal for each set.

Next, plots of $\log k_0$ vs. $\log P_{ow}$ and $\log K_{AM}$ vs. $\log P_{ow}$ were made for the aromatic compounds, to better understand the reasons for the similar results in the aqueous-organic and micellar eluents. A nice straight-line could be drawn through all the data points in the $\log K_{AM}$ plot (Fig. 9.10), but for $\log k_0$, the same dichotomy as in Fig. 9.9 was obtained. It was concluded that the differences in the chromatographic behavior between the phenols and the other aromatic compounds tested, observed in the $\log k$ vs. $\log P_{ow}$ plot could not be attributed to a mobile phase effect, but in all likelihood, to the presence of unhindered silanol groups on the bonded phase surface, as occurs in conventional RPLC. Similar experiences (not shown) were made with CTAB as surfactant. For CTAB, group A was on the left and group B was on the right in the plot of $\log k$ vs. $\log P_{ow}$, and the three plots ($\log k$ vs. $\log P_{ow}$, $\log k_0$ vs. $\log P_{ow}$, and $\log K_{AM}$ vs. $\log P_{ow}$) were similar. Evidently, in this case, the mobile phase effect was important. The interaction of the solutes with a CTAB-coated C18 phase also differed from that of a SDS-coated C18 phase probably due to a change in the surface charge of the stationary phase.

e) QSRR of Ionic Compounds

Many biologically active compounds are ionic at physiological pH. When the degree of ionization is the same for structurally related compounds, the difference in retention is due to the difference in hydrophobicity. However, for ionic compounds with different degree of ionization, linear $\log k$ - $\log P_{ow}$ relationships will not be obtained. In this case, the following model has proved to give good correlations [23-25]:

$$\log k = a \log P_{ow} + b\delta + c \quad (9.30)$$

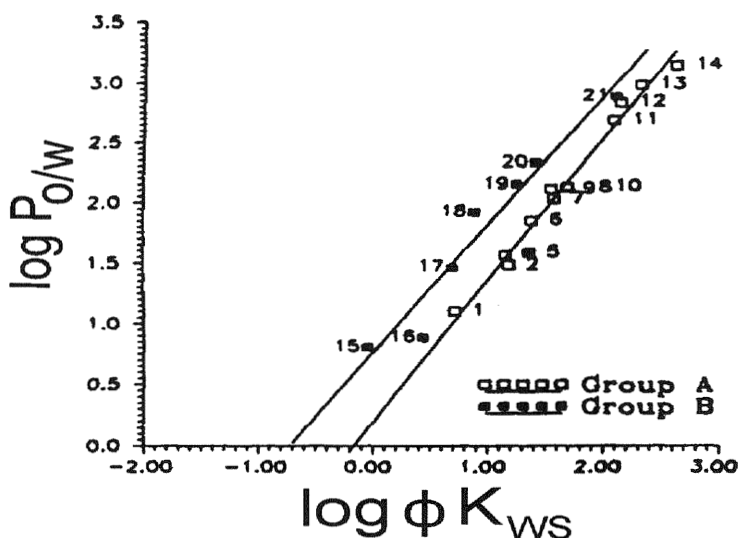
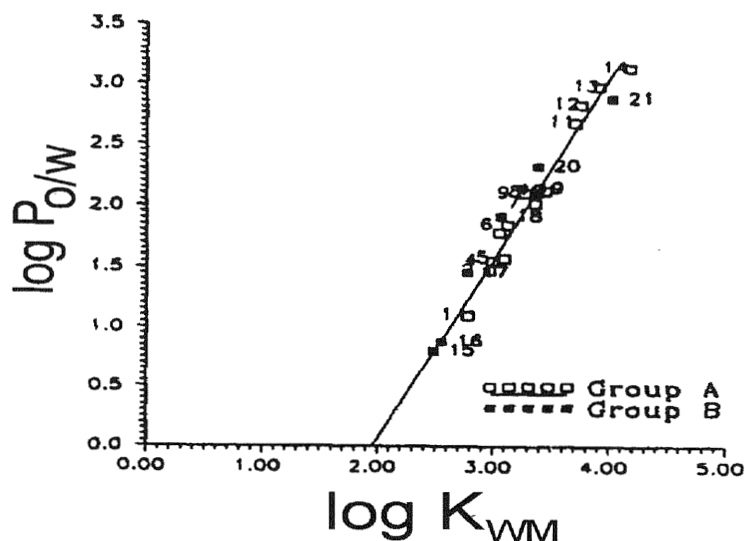


Figure 9.10 Plots of $\log P_{ow}$ vs. logarithm association constants between: water and micelles (top), and water and stationary phase (bottom), using SDS mobile phases with 2% 1-propanol. The compounds are the same as in Figure 9.9. Reprinted from Ref. 15 with permission of Elsevier.

where a and b are fitting coefficients, and δ is the molar fraction of the charged form of the compound, which can be calculated as:

$$\delta = \frac{K_H [H^+]}{1 + K_H [H^+]} \quad (9.31)$$

K_H being the protonation constant. It was found, with a group of catecholamines, that the value of a remained constant with pH. This indicated that the contribution of hydrophobicity to the retention did not vary when the pH of the mobile phase changed [23].

f) Advantages of the MLC Approach to Estimate the Hydrophobicity

MLC provides several advantages for estimating the hydrophobicity of compounds, instead of conventional RPLC with aqueous-organic mixtures. In an MLC system, silanophilic adsorption of surfactant monomers (in equilibrium with micelles) on the alkyl-bonded stationary phase is produced. The stationary phase becomes more hydrophobic and the concentration of residual silanol groups on the silica surface (especially for cationic surfactants) is reduced. In conventional RPLC, the residual activity of alkyl-bonded stationary phases can influence the retention of certain solutes, giving inadequate prediction of hydrophobicity. As a consequence, k_0 could be better than k_w for these predictions. However, for charged solutes eluted with an ionic surfactant, electrostatic interactions will occur. In this case, hydrophobicity predictions using k_0 will fail. Adequate selection of the nature of surfactant and pH of the mobile phase could eliminate this problem.

On the other hand, the environment of the surfactant-modified stationary phase is independent of micelle concentration in the mobile phase (for most surfactants and stationary phases), and similar to that of pure aqueous eluent systems. As a result, the alkyl-bonded stationary phase will have both invariable amphiphilic and anisotropic properties. In contrast, in aqueous-organic RPLC, the composition and structure of the alkyl-bonded phase change with the concentration of organic modifier in mobile phase.

The intercalation of organic solvent in the alkyl-bonded phase plays an important role in influencing retention and selectivity.

Finally, the retention behavior of compounds (nonpolar, polar or ionic) chromatographed with anionic, cationic and nonionic surfactants has been accurately modeled (eq. 9.11). This permits the correct evaluation of k_0 , while large errors in measuring k_w values are frequent. The only problem with k_0 is that it cannot be measured for very hydrophobic compounds owing to the near zero intercept for the $1/k$ vs. $[M]$ plot, although this problem can be improved by choosing a shorter chain-length stationary phase, or mobile phases containing an organic modifier to increase the elution strength.

III.3. *QSRR using other Descriptors*

a) *Prediction of the Retention of PAHs based on Molecular Properties*

A more complex approach in QSRR is the description of the retention behavior of solutes by a multiparameter equation that includes several descriptors related to different structural properties. Chromatographic retention data must be some function of the chemical structure of solutes, stationary phase and mobile phase, all of them mutually interacting. However, there is no general, strict, canonical equation relating the retention to these variables. Even if the stationary and mobile phases in a given chromatographic system remain constant, still a precise quantitative description of the retention of a series of solutes appears problematic, and the difficulties increase with the diversity of the solutes considered. The problem is also not trivial for homologues.

A typical strategy in QSRR studies is to generate a multitude of solute descriptors that are regressed against retention data. The minimum number of descriptors needed to produce an equation yielding the calculated retention data, in satisfactory agreement with the observed values, is selected observing all the statistical rules. The number of descriptors that can be assigned to an individual solute is virtually unlimited. If tens or hundreds of descriptors are used, then most likely, several equations with similar

predictive abilities but consisting of different sets of variables can be derived. Linear regression models showing the dependence between retention and molecular structure make the selection of the optimum conditions for the separation and identification of unknown peaks on chromatograms of multicomponent mixtures possible. However, QSRR studies that are not interpretable in physical terms are not very informative regarding the mechanism of retention.

Rodríguez-Delgado et al. used statistical and principal component analysis to establish some general equations that relate the retention, in MLC, to several molecular descriptors of 17 PAHs eluted with mobile phases of SDS, CTAB and Brij® 35 without alcohol [22], and with methanol, 2-propanol or 1-butanol [26]. PAHs are widely distributed pollutants in the environment and show mutagenic and carcinogenic properties. Therefore, great efforts have been made to develop methods for their quantitation and measurement of hydrophobicity. Nonpolar species possessing polarizable electrons, such as PAHs, have been found to reside near the polar head groups rather than deep within the core of micelles. However, a precise location is impossible to establish, especially due to the fact that many of these solutes have dimensions which are comparable to those of micelles.

The results obtained from the QSRR study indicated that the shape, size and hydrophobicity of PAHs are the dominant molecular characteristics for determining their retention in MLC. The behavior of unsubstituted PAHs was well established using the model:

$$\log k = a \text{ CF} + b \text{ L/B} + c \quad (9.32)$$

where CF is a correlation factor calculated for each PAH as [(number of double bonds) + (number of primary and secondary carbon atoms) - 0.5 for a nonaromatic ring], and L/B is the ratio of the maximalized length-to-breadth of the rectangle enclosing the molecules. The parameter CF is directly related to the size of the molecule and nondirectly to its hydrophobicity, whereas L/B refers to the shape of the molecule. The retention of unsubstituted PAHs increased with both geometric descriptors

CF and L/B. The regression coefficient of the fitted line for methyl-substituted PAHs was however very low, which was explained by the nonplanarity of the molecules due to the methyl group. For these compounds, two new descriptors (selected among 13 starting descriptors subjected to factor analysis) were added to the model. The fraction of nonpolar unsaturated surface area over total surface area (NUSA/TSA) gives information about the electron delocalization along the aromatic rings. The dipole moment (DPMON) was the second descriptor. The retention increased when the NUSA/TSA ratio decreased and when DPMON increased, although the relevance of this descriptor was lower and only acquired importance in describing the electronic properties of isomers. The relevance of these latter descriptors is in agreement with the idea that polarity can play an important role in the retention behavior of PAHs.

The coefficients of the independent variables in eq. 9.32 change with the nature of surfactant and alcohol modifier and their concentrations, through first- and second-degree polynomials. These equations may allow the evaluation of the retention of a solute for any given chromatographic condition.

b) Linear Solvation Energy Relationships

Linear solvation energy relationships (LSER) have also been applied for the evaluation of the retention behavior in RPLC. In LSER, solvent-related properties of solutes, SP (*e.g.*, $\log k$, $\log P_{ow}$ or $\log$ aqueous solubility), are described in terms of solvatochromic parameters in the following general form:

$$SP = SP_0 + \frac{mV}{100} + s\pi^* + b\beta + a\alpha \quad (9.33)$$

where SP_0 is the regression constant, V is the molar volume of solutes related to the solubility behavior, π^* is a measure of solutes' ability to engage in dipolarity/polarizability interactions with the solvent, β the solutes' basicity, and α the solutes' acidity. The coefficients m , s , b and a are related to the chemical nature of the solvent systems. The logarithmic relationships

represent the free energy of transfer of solutes from one phase (e.g., mobile phase or aqueous phase) to another phase (e.g., stationary phase or 1-octanol phase). The term $mV/100$ represents the disfavorable endoergic cavity formation process of separating the solvent molecules to provide a suitably sized enclosure for the solute; $V/100$ instead of V is used to adjust the magnitude of the cavity term within the same range as the other independent variables in eq. 9.33. The term $s\pi^*$ measures the favorable exoergic effects of solute-solvent dipole-dipole and dipole-induced dipole dielectric interactions, and $b\beta$ and $a\alpha$ measure the exoergic effects of hydrogen bonding involving the solvent as a hydrogen bond donor (HBD) acid and the solute as hydrogen bond acceptor (HBA) base, or the solvent as an HBA base and the solute as an HBD acid, respectively.

Yang and Khaledi [27] applied LSER to the evaluation of the retention behavior (k and K_{AM}) of uncharged substituted aromatic compounds of diverse hydrophobicity, using C8 and diphenyl columns, and pure and hybrid mobile phases of SDS and C_{14} TAB with 2-propanol and 1-butanol as modifiers. Zou et al. [28] made a similar study with K_{AM} and P_{ws} , and found high correlations between the retention and solvatochromic parameters of solutes.

The regression coefficients of LSER models between K_{AM} , $\log k$ or k , in MLC, as well as $\log k$ in conventional RPLC with aqueous-organic eluents, and the solvatochromic parameters for a group of 16 aromatic compounds are shown in Table 9.5. One common feature among all LSER models in the table is that the cavity term is the most important factor. For SDS, the correlation was better using $\log k$, whereas for C_{14} TAB, k correlated better with the solvatochromic parameters, as observed previously in MLC for the relationship between k and $\log P_{ow}$ for the same group of compounds. The $a\alpha$ term was positive for solutes binding to cationic micelles, while negative for anionic micelles. This suggested that C_{14} TAB provides a more basic environment to the acidic solutes than SDS. Also, C_{14} TAB micelles are less dipolar (more negative s values) for solutes than SDS micelles.

Table 9.5 LSER Regression in MLC (eq. 9.33) for 16 Aromatic Compounds [27].^a

SP	Eluent	SP ₀	m	s	b	a	r
log K _{AM}	SDS + 3% 2-propanol	0.34	2.81 (0.18)	-0.29 (0.14)	-1.36 (0.13)	-0.35 (0.11)	0.958
log K _{AM}	C ₁₄ TAB + 3% 2-propanol	0.97	2.67 (0.14)	-0.88 (0.10)	-1.44 (0.10)	0.32 (0.08)	0.970
log k	0.08 M SDS + 3% 2-propanol	1.27	1.52 (0.14)	-0.92 (0.11)	-0.78 (0.10)	-0.92 (0.08)	0.970
k	0.08 M SDS + 3% 2-propanol	-5.52	121.9 (9.0)	-40.4 (6.9)	-59.9 (6.4)	-8.6 ^b (5.4)	0.948
log k	0.08 M C ₁₄ TAB + 3% 2-propanol	1.02	1.01 (0.09)	-0.23 ^b (0.13)	-0.76 (0.06)	-0.02 ^b (0.01)	0.943
k	0.08 M C ₁₄ TAB + 3% 2-propanol	0.53	53.9 (2.7)	-8.22 (2.04)	-32.5 (1.9)	1.36 ^b (1.58)	0.975
log k	Methanol-water 40:60 (v/v)	-0.33	3.22 (0.08)	-0.32 (0.06)	-1.73 (0.06)	-0.23 (0.05)	0.994
log k	Propanol-water 40:60 (v/v)	-0.16	1.84 (0.11)	-0.31 (0.09)	-1.43 (0.08)	-0.22 (0.07)	0.976

^a Uncertainties at the 95% confidence level are given.^b Values are not statistically significant at the 95% confidence level.

IV. QUANTITATIVE RETENTION-ACTIVITY RELATIONSHIPS

IV.1. Prediction of Bioactivity of Compounds

The biological activity of many organic compounds, the bio-accumulation of organic pollutants and soil sorption of environmental contaminants, have all been attributed to the hydrophobic character of molecules. Quantitative structure-activity relationship (QSAR) studies play an important role in contemporary drug design, toxicology and environmental monitoring. In QSAR, the biological activity is viewed as a summation of the different

interactions that a compound undergoes both during the transport through biological membranes and in the reaction with the sites of action (receptor). These interactions are assumed to be governed by the chemical structure of the compound. A change in structure can result in a change in biological response.

The partitioning of solute molecules into lipid bilayers and biological membranes is the basis for drug and metabolite uptake, passive transport across membranes and bioaccumulation. For the prediction of the net effects of complex pharmacokinetic and pharmacodynamic processes, the information extracted from diversified data may be more useful than based on individual, one-dimensional hydrophobicity scales. To date, Hansch's hydrophobicity parameter, $\log P_{ow}$, is the most widely used reference to characterize the hydrophobic contribution to the free energy change in the biological response, while the dipole moment and Hammett's σ are used to describe electronic contributions; Taft's steric parameter, molar refractivity and van der Waals volume are often used for size and steric contributions. Another approach in QSAR has been the application of chromatographic techniques, mostly RPLC. The use of chromatographic parameters gives rise to a new field: quantitative retention-activity relationships (QRAR).

IV.2. QRAR Studies by Micellar Liquid Chromatography

a) Use of Micelles as Models for Biomembranes

The 1-octanol-water system is challenged as the only valid hydrophobicity scale. It may not be the best model for studying the partitioning process in biomembranes. Does not the retention in RPLC model better this partitioning? It has been argued that the chemical bonded stationary phase resembles more the hydrocarbon chains of membranes, but the bonding density of almost all commercial columns may be too low to provide a suitable model.

In contrast, micelles have long been recognized as simple chemical models for biomembranes [29]. Indeed, structurally, micelles are more similar to biomembranes than 1-octanol or RPLC stationary phases. Several researchers have demonstrated that the solubilization (or partitioning of solutes) into micelles closely resembles that of lipid bilayers. Both micelles

and biomembranes have amphiphilic properties and are anisotropic media that provide both hydrophobic and electrostatic sites of interaction.

The use of micelles to model biomembranes in QSAR studies has however received little attention. Perhaps the difficulties associated with measuring micelle-water partition coefficients by conventional methods have been the major obstacles in conducting an extensive investigation of the use of micellar systems. The following contributions show that a combination of the unique characteristics of micelles and the capabilities of RPLC in physicochemical studies should be quite useful in QRAR research.

b) Estimation of Phenol Toxicity and Anesthetic Action

Breyer et al. [30] reported interesting results on the capacity of prediction for MLC of the toxicity of a group of 26 p-substituted phenols. The biological activity of these compounds was measured as $\log 1/IGC50$ ($\log 1/C$), where IGC50 is the 50% inhibitory growth concentration of phenols in the culture of *Tetrahymena pyriformis*. A graphical representation of the effect of mobile phase composition on the quality of the predictions is given in Fig. 9.11. Micellar systems gave better prediction of toxicity than conventional RPLC systems with aqueous-organic mobile phases. Addition of 10% 2-propanol to the micellar aqueous phase resulted in a better correlation. Also, a diphenyl column gave a slightly better correlation with toxicity than a C18 column, since the former could better differentiate between the more hydrophobic phenols. The polarizability and configuration of the stationary phase, and the possibility of π - π interactions between solutes and stationary phase may have considerable effect on the retention of more hydrophobic phenols.

The predictive ability of the MLC QRAR model is compared in Fig. 9.12 with a three-variable QSAR model. The predicted value of $\log 1/C$ was calculated from the fitted $\log 1/C$ vs. k and $\log 1/C$ vs. ($\log P_{ow}$, acid dissociation constant pK_a and resonance parameter R) plots, by using the leave-one-out technique (the compound predicted was left out in the derivation of the model). As observed, the QSAR model had difficulties in predicting the toxicity of highly lipophilic phenols, as indicated by the curvature in this region. The results show that a single MLC retention

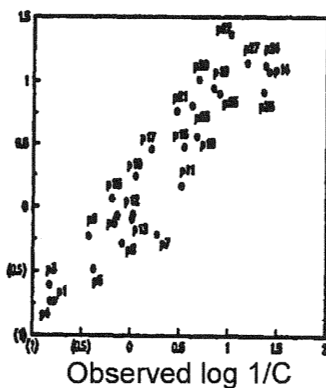
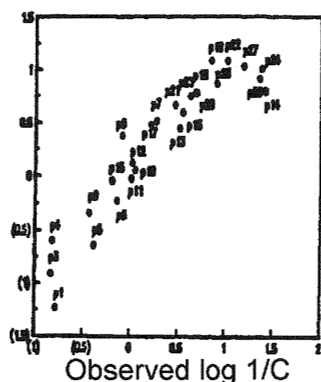
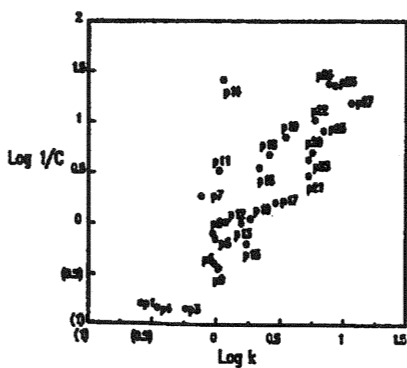
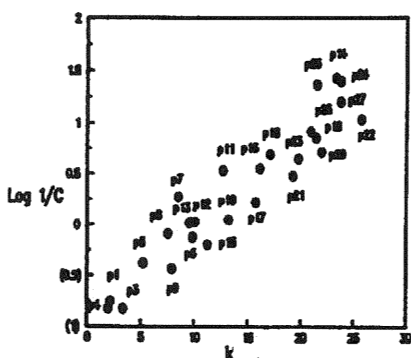
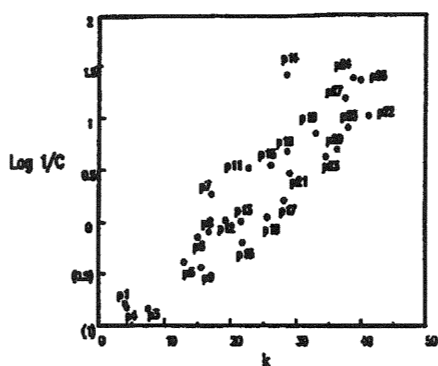


Figure 9.12 Plots of predicted vs. observed $\log 1/C$ for the three-variable QSAR model (with $\log P_{ow}$, pK_a and R as descriptors, see text for meaning) (top, $r = 0.928$), and the one-variable MLC QRAR model (bottom, $r = 0.936$). Reprinted from Ref. 30 with permission of the American Chemical Society.

Figure 9.11 Correlations between $\log 1/C$ and k (or $\log k$), for a series of 26 *p*-substituted phenols eluted with: 0.04 M CTAB (top, $r = 0.917$), 0.04 M CTAB-10% 2-propanol (middle, $r = 0.946$), and methanol-water 40:60 (v/v), (bottom, $r = 0.812$). A diphenyl column was used. Reprinted from Ref. 30 with permission of the American Chemical Society.

parameter is capable of describing the bioactivity of phenols, while three structural descriptors conventionally used in QSAR are needed to achieve a similar correlation. Of course, the addition of other structural parameters to k would further improve the correlation with $\log 1/C$.

Since the toxicity of phenols was measured at a physiological pH of 7.4, the good correlation between $\log 1/C$ and k obtained at pH 7 is not surprising. It is possible that because the micellar system closely mimics the biological system, the same form (molecular, ionized or partially ionized) of the compound responsible for a particular biological response also exists in the chromatographic column. In situations like this, QRAR is a suitable alternative to QSAR since measuring MLC retention is much easier than measuring different molecular descriptors needed to build the QSAR model.

Good relationships between the retention in MLC and some biological activities of local anesthetics (bupivacaine, lidocaine, mepivacaine, prilocaine, procaine and tetracaine), such as anesthetic potency, concentration of compound that produces an effect similar to a reference concentration of cocaine, duration of the action, toxicity and time taken to eliminate half the drug present in the body, have also been reported [24]. Some anesthetic actions of barbiturates also correlated well with the retention: minimum effective hypnotic dose in rabbits, molar drug concentration necessary to reduce cell division, and molar drug concentration required to reduce 50% the inhibition of oxygen respiration on the brain of a rat *in vitro* [25].

c) *Correlation between the Retention of Diuretics and their Site of Action within the Kidney*

A final example of the capability of MLC to characterize bioactive substances is the report of Medina Hernández et al. [31], related to the action of diuretics in the nephron. A wide variety of compounds with different chemical structures have been described to act as diuretics. These compounds enhance renal excretion of water and electrolytes through interference of the mechanisms of ionic transport all along the nephron, which is constituted of a glomerule and a long tubule where a filtration process takes place (Fig. 9.13). Each individual segment in the nephron

(proximal tubule, loop of Henle, distal convoluted tubule and collecting duct) has a different function, and different diuretics possess specific sites of action. Even more, the action of a diuretic produces a characteristic profile regarding the excretion of water and electrolytes, and such profile may suggest precisely its site of action within the nephron. To define these sites of action in vivo and in vitro studies are usually performed in humans and laboratory animals. According to their action, the compounds are classified as high, intermediate and low efficacy diuretics.

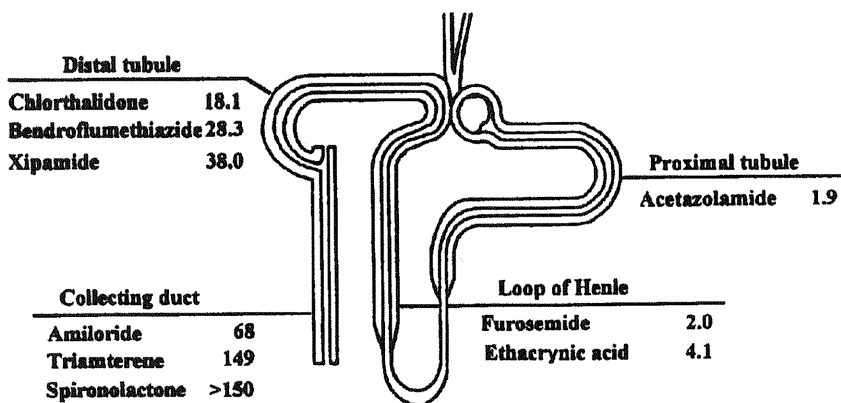


Figure 9.13 Sites of action along the nephron and retention factors for some diuretics eluted with a 0.03 M SDS mobile phase at pH 7.

The therapeutical action of diuretics has been attributed to their hydrophobic character, among other properties. As commented, the therapeutical and physiological classifications of diuretics are correlated. The site of action for high efficacy diuretics is the loop of Henle, for intermediate efficacy diuretics is the distal tubule and for low efficacy diuretics is the proximal and distal tubules and the collecting duct. It has been shown that MLC with SDS mobile phases and C18 columns offers a scale for hydrophobicity, which leads to a further correlation between the retention and the sites of action of diuretics within the nephron. The site of action of those diuretics showing the lower retention in MLC is the proximal

tubule (acetazolamide) and the loop of Henle (loop diuretics: bumetanide, furosemide, ethacrynic acid), for those with an intermediate retention is the distal tubule (thiazides: benzothiazide, chlorthalidone, bendroflumethiazide, dihydrochlorothiazide, xipamide), and for those with a long retention is the collecting duct (potassium sparing diuretics: amiloride, triamterene, spironolactone). However, hydrochlorothiazide, an intermediate diuretic, showed a retention similar to high efficacy diuretics (acting in the loop of Henle). This behavior may be in accordance with the fact that hydrochlorothiazide acts in other sites within the nephron. Thus, it competes with uric acid for the secretion of organic acids at the level of the proximal transporter system.

The chromatographic retention of diuretics decreased as the concentration of SDS in mobile phase increased, which made retention times closer to each other. Among the micellar mobile phases, 0.03 M SDS gave the best correlations between retention factors and site of action. It is interesting to note that the correlations hold in spite of the largely diverse chemical structures of diuretics. The order of retention attained with other mobile phases in conventional RPLC systems was checked to be completely different from that of MLC, and a correlation with regard to physiological properties could not be drawn.

MLC should be developed further as a means of characterizing bioactive substances. The chromatographic system is dynamic in nature, which suggests the possibility of using MLC to mimic physiological systems such as the nephron, where both hydrophobic and electrostatic interactions, as well as kinetic phenomena can be important.

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10

Analytical Use of Micellar Liquid Chromatography

I. INTRODUCTION

The first applications of Micellar Liquid Chromatography (MLC) in analytical chemistry were published in 1984. The reports showed the interest of using micellar mobile phases in the analysis of plasma [1], proteins [2] and pesticides [3]. However, the research on MLC has been largely dedicated to the retention mechanism inside the chromatographic system and the relationships between the retention behavior and hydrophobicity of solutes. Indeed, micellar mobile phases are quite interesting from a mechanistic standpoint, but some practical analytical utility had to be demonstrated before they could be considered as an alternative to the more traditional aqueous-organic mobile phases.

In the nineties, the number of analytical procedures in MLC proposed by several authors increased, especially in the field of the determination of drugs in physiological fluids and pharmaceutical preparations. The analysis of other types of samples was less frequent. This chapter shows the experimental procedures to be followed in handling micellar mobile phases for the analysis of several types of samples, with the exception of physiological fluids, which will be considered in the next chapter, owing to the particular characteristics and interest of these samples.

A practical requirement of any micellar mobile phase is its compatibility with the sample matrix. Micellar eluents can be used to dissolve samples or extract analytes. Surfactants can cosolubilize nonpolar and polar compounds, derivatization reagents and products. They can also induce favorable shifts in the equilibrium constants and spectral properties, inhibit undesirable reactions, stabilize reaction intermediates, and expedite reactions by means of micellar catalysis [4, 5]. Other potential advantages

of micellar eluents include a much lower cost than traditional liquid chromatographic grade solvents, adjustable solvent strength and lower ignitability and toxicity.

Surprisingly, almost all the applied work in MLC to date appears to have involved only ionic micellar mobile phases composed of either anionic sodium dodecyl sulfate (SDS), or cationic hexadecyltrimethylammonium bromide or chloride (CTAB or CTAC), and dodecyltrimethylammonium bromide (DTAB). Reports on the use of uncharged nonionic surfactants, such as Neodol® 91-6 and polyoxyethylene(23) dodecanol (Brij® 35) are exceptions.

It is believed that when micellar mobile phases are used, the chromatographic columns deteriorate very easily. However, with an adequate experimental methodology (see Chapter 4), no apparent degradation of the chromatographic performance will occur. Hundreds of injections can be made without modification of the retention of the compounds or pressure buildup in the chromatographic system. In our laboratories, we have used the same columns for a year or even longer (at least 600 injections), without any apparent deterioration.

The chromatographic system should comprise a precolumn located between the pump and the injector, packed with silica gel, in order to saturate the mobile phase with silicic acid, thus increasing the lifetime of the analytical column. Caution must be taken to insure that the steady-state condition required for reproducible chromatography is reached. Before the sample is injected into the chromatograph, the system must be equilibrated with the micellar mobile phase. Special care is required to work with micellar mobile phases such as those given in Chapter 4 (Section IV).

Finally, it should be reminded that for micellar solutions of ionic surfactants, the temperature of the column should always be above the Krafft point (*e.g.*, 15°C for SDS), in order to avoid clogging and possibly ruining the column. Nonionic surfactants, instead, have a cloud point temperature at which phase separation occurs (see Chapter 2). In this case, all chromatographic work should be conducted below this temperature. However, this depends on the concentration of surfactant and is usually very high (*e.g.*, approximately 100°C for aqueous 1-6% Brij-35).

II. MICELLAR CHROMATOGRAPHY OF PROTEINS AND ENZYME ACTIVITY

II.1. Determination of Proteins

a) Use of Nonionic Surfactant in the Separation of Proteins

Reversed-Phase Liquid Chromatography (RPLC) is an important tool in protein chemistry. Examination of sorption isotherms revealed that alcoholic buffers did not desorb proteins near physiological pH in RPLC systems, while buffers containing a poly(ethoxy alcohol) surfactant did not desorb protein at pH 2, but they did at pH 7 with concentrations of surfactant apparently well above the critical micellar concentration (cmc) [2]. It has been proposed that a necessary condition for the desorption of a protein from a surface is that the surface tension of the solvent falls between that of the protein and the surface [6]. This condition is fulfilled for many proteins with surfactant solutions near conditions of physiological pH and ionic strength. Therefore, it was expected that separations of proteins could be achieved in these conditions.

It was effectively found that a mobile phase of Neodol® 91-6, a nonionic surfactant, separated proteins on a reversed-phase column at neutral pH [2]. Neodol® 91-6 is a blend of C9, C10 and C11 primary alcohols with an average of 6 moles of ethylene oxide per mole of alcohol. Owing to the several chain-lengths and degrees of ethoxylation, this surfactant has a cmc range rather than a fixed value. When individual proteins were injected into a C8 column, their retention volumes fell into three broad groups, depending on the isocratic concentration of Neodol 91-6 required for elution. Table 10.1 shows these groups (low, intermediate and high retained proteins), together with the molecular weights, isoelectric points, and average hydrophobicities of the proteins.

The chromatographic behavior was rationalized in terms of favorable van der Waals attraction of the proteins by the nonionic micelles and the reduced surface tension provided by the aqueous micellar mobile phase. However, little correlation of the retention with the molecular weight was observed, although the most strongly retained proteins were of low

molecular weight. The accessibility of these proteins to a larger percentage of the packing's surface area may account for the observed retention. Surface charge also has minimal effect, as indicated by the occurrence of basic proteins in all three groups. The average hydrophobicity has been correlated with protein properties, such as solubility, aggregation phenomena, and thermal stability, but no direct or inverse correlation was observed in this case.

Table 10.1 Properties of Proteins of Low, Intermediate and High Retention in Surfactant-Reversed Phase System (*pH* 7) [2].

Compound	Molecular Weight $\times 10^{-3}$	Isoelectric Point (pI)	Average Hydrophobicity $\times 10^{-3}$
Low Retention			
Ovalbumin	45	4.6	1.11
Catalase	58	5.7	
Carbonic anhydrase	32	7.3	1.06
Ferritin	800	5.0	
Apo ferritin	24		1.05
Intermediate Retention			
Bovine serum albumin	65	4.8	1.12
Thyroglobulin	335	4.6	
Chymotrypsinogen	23	9.2	1.05
Ribonuclease	14	9.6	
High Retention			
Lysozyme	14	11.0	0.97
Cytochrome <i>c</i>	13	10.0	1.11
β -Lactoglobulin	18	5.2	1.23

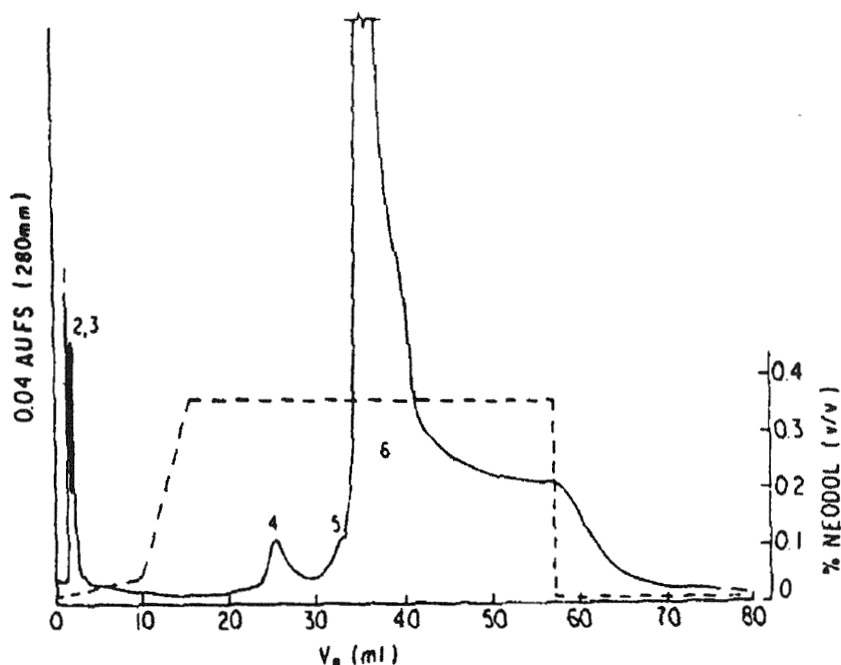


Figure 10.1 Gradient micellar chromatography of protein mixture: 1. ovalbumin, 2. bovine serum albumin, 3. thyroglobulin, 4. chymotrypsinogen, 5. β -lactoglobulin, 6. lysozyme. Column: Supelcosil LC-8; mobile phase: Neodol 91-6 in 0.05 M phosphate at pH 7. Reprinted from Ref. 2 with permission of the American Chemical Society.

Unlike chromatography of most low molecular weight solutes, where increases in micelle concentration produce reductions in retention, small decreases of surfactant concentration caused exponential increases in protein retention. Therefore, to obtain adequate separations of mixtures of selected proteins, shallow gradients were employed. Fig. 10.1 illustrates the potential of micellar mobile phases for separating components with a wide range of properties, as is commonly the case for biological isolates. For some purposes, isocratic elution gave sufficient separation, although caution was taken to assure that no protein remained sorbed on the support. An example of separation of proteins is given in Fig. 10.2.

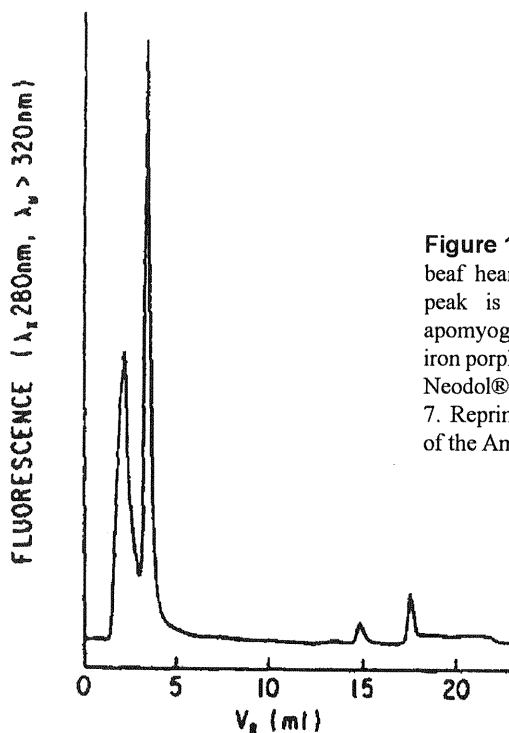


Figure 10.2 Chromatogram of a beef heart myoglobin preparation. First peak is myoglobin, second peak is apomyoglobin (tentative), later peaks are iron porphorins (tentative). Mobile phase: Neodol® 91-6 in 0.05 M phosphate at pH 7. Reprinted from Ref. 2 with permission of the American Chemical Society.

b) Determination of Recombinant Human Growth Hormone

A method was described for the RPLC determination of recombinant methionylaspartyl-human growth hormone (MD-HGH) in *Escherichia coli* (*E. coli*) fermentation broth [7], which utilizes mobile phases containing the anionic surfactant SDS and 1-propanol, under micellar conditions. A C4 column was used at 60°C for the separation. The methodology is directly applicable to the analysis of samples solubilized via sulfitolysis in the presence of SDS, and offers superior resolution in comparison with chromatography in the absence of the surfactant.

The analysis of heterologous proteins in recombinant hosts, such as *E. coli*, presents many challenges to the analytical biochemist. The cells must be lysed and the inclusion bodies solubilized prior to quantification. Cell lysis and protein solubilization can be accomplished chemically through the use of SDS. As the proteins in inclusion bodies can exist as a distribution of forms, such as covalent and noncovalent polymers, it is crucial to convert the target protein into a single molecular entity prior to analysis. This can be achieved by unfolding the proteins and disrupting the inter- and intramolecular disulfide bonds via reduction or sulfitolysis. The complexity of the matrix adds to the difficulty in the determination of the recombinant protein, as both the whole cell and the inclusion bodies can also contain nucleic acids, salts, lipids, and other host molecules, in addition to proteinaceous material.

In fact, the employment of ionic surfactants, such as SDS, had traditionally been avoided in the chromatographic analysis of proteins owing to the generation of a strongly denaturing environment. However, for the determination of recombinant proteins in fermentation broth, the characteristics of these surfactants are useful for the enhancement of the solubility of unfolded denatured proteins, the elimination of irreversible adsorption on the stationary phase and the facilitation of unique separation selectivity. In the procedure reported for the determination of MD-HGH, the sulfitolysis solubilization reagent was prepared with SDS, Tris, anhydrous sodium sulfite, anhydrous potassium tetrathionate and disodium ethylenediaminetetraacetate dihydrate at pH 8.5-8.7. The sulfitolysis process was complete within 6-8 hours under these conditions at room temperature. Next, the reaction was quenched and the solubilized protein stabilized by adjusting the pH of the solution to 5.8-6.2 with maleic acid.

The optimum mobile phase pH for the determination of MD-HGH appeared to be near 6.4. The recovery, evaluated by spiking a known volume of fermentation broth sample with a nonsulfitolyzed MD-HGH standard solution at four levels ranging from 100 to 800 $\mu\text{g/ml}$, amounted in average 103.5%. This indicated complete extraction of the sulfitolyzed protein from the broth.

II.2. *Evaluation of Folylpolyglutamate Hydrolase Activity*

Folylpolyglutamate hydrolase specifically catalyzes the hydrolytic cleavage of peptide bonds involving the γ -carboxyl group of glutamic acid. Since folates in food occur mainly as polyglutamates which are poorly absorbed, much of the interest in these enzymes has centered on their role in the bioavailability of dietary folates. However, folates may also play a role intracellularly in the control of one-carbon metabolism. Intracellular folates are almost entirely polyglutamyl derivatives and changes in polyglutamate chain-lengths are known to have dramatic effects on the kinetics of a number of folate-requiring enzymes.

Early microbiological assays of folylpolyglutamate hydrolase were sensitive, but also lengthy, hard to reproduce, subject to interference and lacked of specificity. The proposal of a more simple and sensitive method for the quantitative determination of the activity of this enzyme in crude tissue extracts was thus attractive. The new procedure was based on RPLC separation of folate analogue mono- and polyglutamates on a C18 column, using SDS in water as the mobile phase under isocratic conditions [8]. Interfering substances in tissue extracts were removed by gel filtration on centrifugally-eluted minicolumns of Sephadex G-25, prior to incubation of polyglutamate substrate with tissue extract hydrolase. Reactions were terminated by denaturation of the enzyme in SDS, which subsequently served as the micellar solvent system for the chromatographic separation.

Diglutamates are usually the substrates of choice for the quantitation of hydrolase activity since, unlike longer chain-length congeners, they have only a single γ -glutamyl peptide bond. Thus, the product of hydrolysis cannot subsequently become a substrate. Also, the analysis is not complicated by the endopeptidase activity associated with some hydrolases. The procedure was performed with 5,8-dideaza-isopteroyl- γ -glutamyl-L-glutamic acid (IAHQ-Glu) because of its relative ease of preparation and availability. Fig. 10.3A shows the complete separation of IAHQ-Glu from the peak at the solvent front. Progressive hydrolysis of the substrate is shown in Figs. 10.3B-D. The baseline resolution of the substrate from the product, IAHQ, permits quantitative assessment of hydrolysis rate.

The assay was linear over a period of at least 2 h. Moreover, the amount of product formed was directly proportional to the amount of added

extract. The results indicated that the method was suitable for the determination of folylpolyglutamate hydrolase activity in cell-free extracts from different mouse tissues, as well as extracts from a wide variety of sources.

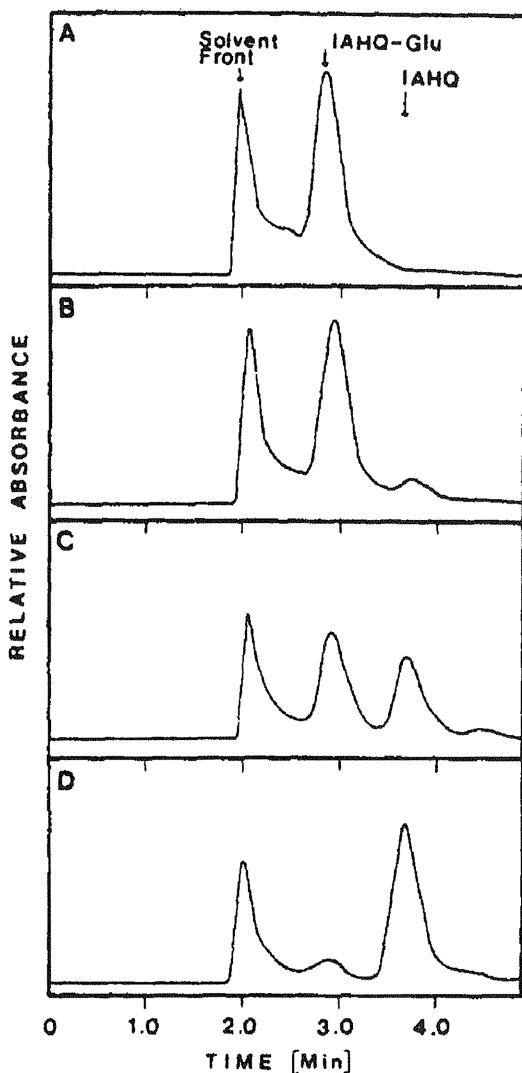


Figure 10.3 Hydrolysis at 37°C of IAHQ-Glu by crude extracts from mouse kidney. Hydrolysis time: 0 min (A), 15 min (B), 60 min (C), and 120 min (D). Reactions were stopped by introduction of SDS, before being chromatographed isocratically with 0.2 M SDS. Reprinted from Ref. 8 with permission of Elsevier.

The micellar solvent system used in this assay has several advantages, in addition to rapid separation: SDS is an effective-denaturing agent and, as such, can be used to stop reactions without the need for protein precipitation with trichloroacetic acid or heating. Since SDS also has a unique solubilizing power, it can be used for direct injection of concentrated protein solutions into the RPLC system, without time-consuming steps to remove protein precipitates or extraction of folate analogues. Therefore, the assay is simple, rapid, inexpensive, and applicable to crude hydrolase preparations.

III. CONTROL OF PHARMACEUTICAL PREPARATIONS

Micellar mobile phases can replace, in many instances, conventional aqueous-organic mobile phases in the control of pharmaceutical preparations with good results. A belief exists that the analytical procedures using micellar eluents are inferior, owing to the frequent low efficiencies of the chromatographic peaks. This is not always the case, as was demonstrated in a comparative study of the performance of RPLC with micellar and aqueous-organic mobile phases, in the analysis of pharmaceuticals containing β -blockers [9].

In MLC analysis of pharmaceutical preparations, the samples are usually treated with a micellar solution. The drugs are easily extracted in this medium, which produces an important reduction in the time employed in the preparation of the sample. The solutions of the pharmaceuticals can be injected into the chromatograph without any other treatment than filtration.

Some features of analytical procedures developed for MLC are explained below, including sample preparation, derivatization of the drugs and optimization of the chromatographic separation.

III.1. Sample Preparation

Analytical procedures have been reported for the MLC analysis of pharmaceuticals presented as tablets, pills, capsules, gels, drops, ampoules, sprays, suspensions and oily injectable doses. Although the drugs are easily solubilized in a micellar solution, the excipients are frequently not soluble in this medium. It may also be convenient to treat, first, the pharmaceuticals with a small amount of ethanol and to add, afterwards, a micellar solution to assure the extraction of the drug. In any case, filtration of the solutions is required previously to its injection in the chromatograph. This operation can however be performed directly on the autosampler vials (*e.g.*, through Teflon 0.45 μm membranes). The recommended treatments for the different types of pharmaceutical samples are the following:

Tablets and Pills. Five to ten tablets or pills should be weighed, powdered and homogenized in a mortar, a portion taken, weighed and dissolved in 0.05-0.1 M SDS using an ultrasonic bath. Water can be used for dilution, but the last dilution should preferably be made with the solution used as mobile phase, in order to reduce the noise at the beginning of the chromatogram when the drug solution is injected.

Capsules. A similar procedure should be followed with the contents of capsules, whose weight can be determined by the difference between the weight of the filled and empty capsules. The capsules should be carefully cleaned to obtain an accurate weight of the capsule contents. For some pharmaceuticals, the capsules can be dissolved in the micellar medium without opening. In this case, a more complex chromatogram will be obtained due to the presence of the capsule materials (acid gelatine and pigments).

Gels. An adequate amount should be weighed and dissolved in 0.05-0.1 M SDS.

Drops, Ampoules and Sprays. An aliquot of the solution should be taken and mixed with 0.05-0.1 M SDS solution.

Suspensions. Suspensions should be mechanically stirred and introduced in an ultrasonic bath in alternate periods, before taking the sample for analysis. An aliquot of the homogenized suspension should be dissolved with 0.05-

0.1 M SDS, using an ultrasonic bath. Clear micellar solutions are easily obtained.

Oily Injectable Doses. Most often the individual dose is contained in a breakable vial. The oily contents of two or three vials can be mixed, an aliquot is taken and a comparable volume of 0.1 M SDS is added. A homogeneous emulsion is formed by alternating 5 min periods of mechanical stirring and ultrasonic bath. The emulsion should be diluted with 0.1 M SDS to obtain a clear solution for analysis.

III.2. Precolumn Derivatization

The modification of the polarity of some drugs through the formation of derivatives may be convenient to increase their retention in the chromatographic column, or enhance the selectivity of the separation in the analysis of mixtures of drugs in complex matrices. The absorption bands of the analytes are shifted to longer wavelengths upon chromogenic derivatization and this can facilitate their detection. Examples that make use of the advantages of precolumn derivatization have been reported in MLC for the determination of sulfonamides and amino acids. The derivatization reactions were readily performed in a micellar medium of SDS, leading to rapid and simple procedures to control the compounds in pharmaceutical preparations.

a) Determination of Sulfonamides

Diazotization and coupling with the Bratton-Marshall reagent (*N*-(1-naphthyl)ethylenediamine dihydrochloride, NED), combined with spectrophotometric measurement, is perhaps one of the most popular procedures for the determination of arylamines. In the analytical protocols, the amine group is diazotized with sodium nitrite, the excess nitrite eliminated with sulfamic acid, and the diazonium ion produced coupled with NED to form an azo dye (Fig. 10.4). The azo dyes show, in acid medium, an absorption maximum close to 550 nm and a high molar absorptivity ($40,000\text{--}50,000\text{ mol}^{-1}\text{ l cm}^{-1}$). The maximum wavelength shifts to 490 nm at $\text{pH} > 4$ and shows lower absorptivity.

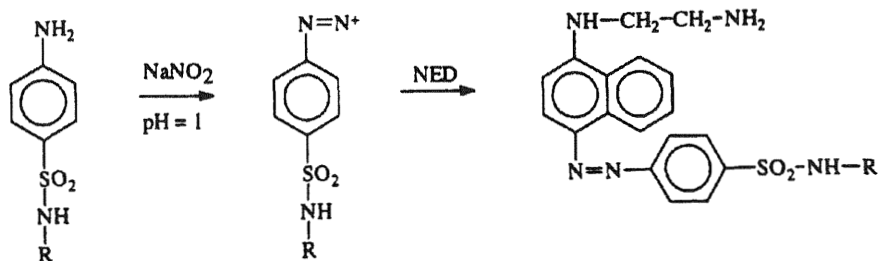


Figure 10.4 Diazotization of sulfonamides and coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride.

It has been demonstrated that these spectrophotometric analytical procedures are largely improved when performed in an SDS micellar medium, where the coupling reactions of the diazotized arylamines with NED are positively catalyzed (the reactions are almost instantaneous), and the protonation constants of the azo dyes are shifted to higher pH values [10]. In nonmicellar solution, diazonium ions are formed at $\text{pH} < 1$, whereas $\text{pH} > 2$ is necessary for coupling at a reasonable speed. A further modification of pH is usually made in order to measure the absorbance of the azo dyes in their protonated forms. In an SDS micellar medium, the combined effects of micellar catalysis of the coupling reaction and the earlier protonation of NED azo dyes make these pH changes unnecessary. With SDS, the coupling and measurement steps can be carried out in a 0.06 M HCl solution, which results from the addition of the coupling reagent to the 0.15 M HCl solution used to diazotize the arylamines.

The pharmaceutical industry commercializes a great variety of formulations that contain sulfonamides, which are used as antibacterial agents in medicine and veterinary practice. When some sulfonamides were directly chromatographed with a 0.1 M SDS mobile phase at pH 7, the drugs eluted with the void volume. At pH 3, broad peaks with retention factors, k , in the 3-4.5 range were observed. In these conditions, the analysis of formulations containing a sulfonamide and other drugs was not possible. The formation of the azo dyes of sulfonamides increased the retention of the

drugs, and permitted the use of a micellar eluent of SDS and 1-pentanol of high elution strength. This improved the selectivity of the determinations [11]. The azo dyes formed immediately and were stable during several days, even when exposed to light and oxygen, except sulfacetamide. The degradation of this azo dye was observed by the diminution of the chromatographic peak at 4-5 min, and the appearance of a second peak at a shorter retention time. If the solution of the sulfacetamide azo dye was injected before 2 h from its formation, only one peak was observed.

b) Determination of Amino Acids

The chromatographic analysis of amino acids with spectrophotometric detection usually requires the formation of derivatives, because of little absorption of UV light above 210 nm. Precolumn derivatization is usually preferable. *o*-Phthalaldehyde (OPA) is the derivatization reagent that probably has the best characteristics. It reacts with primary amino groups in the presence of a thiol at pH 9.5 and room temperature to form 1-alkylthio-2-alkyl substituted isoindoles (Fig. 10.5). The derivatives show maximum absorption at 335 nm and are highly fluorescent, with excitation wavelength at 340 nm and emission at 445 nm. Mercaptoethanol has been more extensively used than other thiols for the derivatization, but the OPA-mercaptoethanol isoindoles are unstable. The stability of isoindoles is improved when *N*-acetyl-L-cysteine (NAC) is used instead of mercaptoethanol [12].

Proteic primary amino acids are found in diverse pharmaceuticals, such as vitaminic and dermatological preparations, and appetite stimulants. Other formulations contain an amino acid in the excipient. Thus, glycine is used to increase the rate of dissolution of acetylsalicylic acid, improving its action and stomachic tolerance. A 0.05 M SDS-3% 1-propanol eluent was used to analyze pharmaceutical formulations containing glycine, lysine, methionine and threonine, and a number of other components, after OPA-NAC precolumn derivatization [13]. The change of pH produced a large variation in the retention of the amino acid derivatives as a result of the protonation of their carboxylate group. Maximum resolution and adequate retentions were achieved at pH 3.

The derivatization was performed by mixing an aliquot of the amino acid solution with equimolar OPA-NAC reagent (OPA-NAC:amino acid

molar ratio ≥ 10). The concentration of OPA was below 10^{-3} M, since the isoindoles are less stable at increasing OPA concentration. The reproducibility was good if the injection into the chromatograph was made one minute after mixing the reagents.

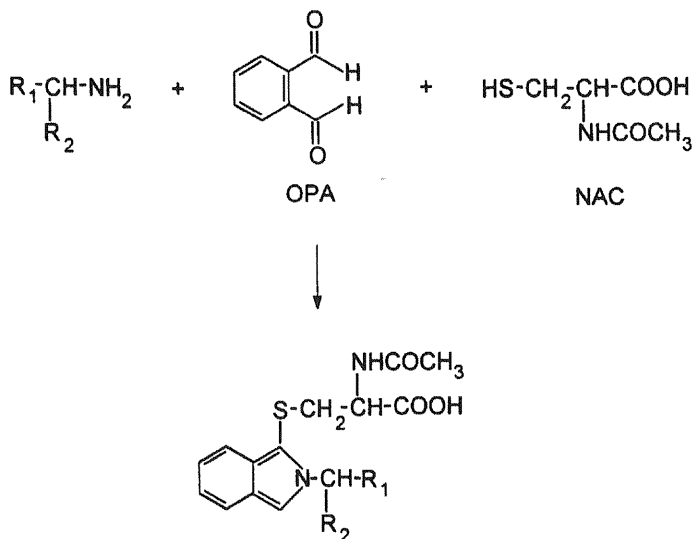


Figure 10.5 Derivatization of amino acids with OPA and thiol to form 1-alkylthio-2-alkyl substituted isoindoles.

III.3. Optimization of Mobile Phase Composition

Several variables should be considered in the development of an MLC procedure: the nature of surfactant and modifier, their concentrations, and pH. When a surfactant solution is used as mobile phase, the retention of solutes can be adequately controlled through the addition of a small amount of alcohol, and through variation of pH. The alcohol usually also improves the efficiency of the chromatographic peaks. Other variables that affect the retention and efficiency are temperature and ionic strength. However, most procedures are performed at room temperature, and the ionic strength is given by the combination of the surfactant and buffer in the mobile phase, and is not studied as a separate variable.

The report commented above for the determination of sulfonamides in pharmaceuticals [11] is a useful example of the development of an analytical procedure, where a sequential optimization is made. Next, the development of a procedure for the analysis of mixtures of β -blockers and diuretics [14] will show the usefulness of the interpretive optimization strategy shown in Chapter 8, which was assisted by the software *MICHROM* (see Appendix I).

a) Sequential Optimization Strategy

The first procedure was developed for the determination of several sulfonamides (sodium sulfacetamide, sulfadiazine, sulfaguanidine, sulfamethizole, sulfamethoxazole, and sulfanilamide), in different pharmaceutical preparations (tablets, pills, capsules, drops, and suspensions), after azo dye formation. The retention of the azo dyes was excessive (analysis times longer than 40 min) with pure micellar SDS mobile phases. The retention times were still high (> 25 min) after addition of 1-propanol as modifier. Therefore, an alcohol giving a higher elution strength, such as 1-pentanol, was preferred.

In an anionic micellar solution of SDS, the primary amine group of sulfonamide azo dyes is protonated in very weak acid media, whereas the protonation of the aryl-alkyl secondary amine group, in *para* position with respect to the azo bridge (Fig. 10.4), takes place usually in the 3.5–4.5 pH range, one to two pH units higher than in a nonmicellar solution [10]. Thus, in the presence of SDS, the single and double charged cationic forms of the azo dyes dominate at $\text{pH} < 9$ and $\text{pH} < 4$, respectively. The higher retention obtained at decreasing pH indicated that the solute interacted more strongly with the anionic modified stationary phase than with the anionic micelles in the eluent. The stronger interaction also decreased the efficiency of the chromatographic peaks. Thus, pH 7 was selected for the mobile phase.

When a hybrid SDS-1-pentanol mobile phase was used, an increasing concentration of SDS gave lower retention, but also lower efficiency. A mobile phase containing a low concentration of SDS was thus preferable. On the other hand, an increasing amount of 1-pentanol gave lower retention, together with increased efficiency. Finally, a 0.05 M SDS-2.4% 1-pentanol mobile phase was selected. With this mobile phase, the sulfonamide azo dyes were eluted in less than 15 min, following the elution

order (k in parentheses): sodium sulfacetamide (4.5), sulfamethizole (5.0), sulfaguanidine (9.1), sulfamethoxazole (10.5), sulfadiazine (11.8), and sulfanilamide (12.1). For the analysis of the most retained drugs, another eluent of higher elution strength was also used.

Table 10.2 shows the composition, recoveries and reproducibilities obtained in the analyses of several pharmaceuticals containing the sulfonamides. The analyses were performed by derivatizing five aliquots of the dissolved pharmaceuticals. The recoveries with respect to the compositions given by the manufacturers were usually close to 100%. The accompanying compounds did not give any peak at the detection wavelength. These results are given as an example of the good performance of an MLC procedure applied to the control of pharmaceuticals.

b) Interpretive Optimization Strategy

Several pharmaceutical formulations that contain a β -blocker, together with one or two diuretics, are commercialized. Some of these preparations also include the vasodilator hydralazine. The combination of the three types of drugs has an additive effect in the treatment of hypertension, producing the diminution of arterial pressure through diverse mechanisms. The simultaneous administration of these drugs is recommended, when an appropriate control of hypertension is not possible with each of them separately.

There are only a few reports on chromatographic procedures, where combinations of a β -blocker and a diuretic are analyzed using the same aqueous-organic mobile phase for both drugs. One example is a procedure recommended in the USP-XXII Pharmacopeia [15] for combinations of metoprolol and hydrochlorothiazide in tablets, where both sample preparation and chromatographic conditions are different for each drug. Sample preparation is especially tedious for metoprolol, since it involves dissolution in HCl with sonication and heating, filtration and triplicate extraction with chloroform, elimination of the organic solvent and redissolution.

The possibility of using the same micellar mobile phase for the analysis of pharmaceuticals, where the β -blockers atenolol, metoprolol and oxprenolol, the diuretics amiloride, bendroflumethiazide, chlorthalidone and

Table 10.2 Analysis of Pharmaceutical Formulations Containing Sulfonamides. Contents Declared by the Manufacturers, Recoveries and Repeatabilities [11]

Formulation	Composition	Recovery %	RSD (%) n = 5
Amidrin-Bio (Fardi, Barcelona)	Per 1 ml drops: sodium sulfacetamide (50 mg), chloramphenicol (5 mg), phenylmethylaminopropanol chlorhydrate (10 mg), trichloroisobutanol (4 mg), excipient	100.1	4.3
Celestone S (Schering-Plough, San Agustín de Guadalix, Madrid)	Per 100 ml drops: sodium sulfacetamide (10 g), betamethasone (100 mg), excipient	98.3	3.6
Visu-blefarite (Merck Sharp & Dohme, Chibret, Alcalá de Henares, Madrid)	Per 1 ml drops: sodium sulfacetamide (100 mg), betamethasone (1.5 mg), tetrazolium phosphate (0.5 mg), excipient	100.8	0.8
Angileptol (Sigma-Tau, Alcalá de Henares, Madrid)	Per tablet: sulfaguanidine (50 mg), tirotricine (1 mg), enoxolone (3 mg), benzocaine (4 mg), sodium saccharin (3 mg), saccharose (300 mg), excipient	101.8	1.8
Bucodrin (Fardi, Barcelona)	Per tablet: sulfathiazole (100 mg), ethacridine (2 mg), ephedrine (3 mg), saccharose (1.8 g), excipient	98.9	5.2
Micturol sedante (Boots Pharmaceuticals, Alcalá de Henares, Madrid)	Per pill: sulfamethizole (125 mg), phenazopyridine chlorhydrate (50 mg), starch (3.25 mg), saccharose (220 mg), lactose, excipient	104.0	4.7

Table 10.2 (continued).

Formulation	Composition	Recovery %	RSD (%) n = 5
Bio-Hubber (ICN Hubber, Corberà de Llobregat, Barcelona)	Per tablet: sulfadiazine (30 mg), neomycin sulfate (25 mg), bacitracin (100 U.I.), estreptomycine sulfate (50 mg), pectin (50 mg), sodium menadione bisulfite (20 mg), nicotinamide (20 mg), saccharin (10 mg), starch (50 mg), lactose, excipient	102.0	2.1
Amidrin (Fardi, Barcelona)	Per 1 ml drops: sulfanilamide (4 mg), ephedrine chlorhydrate (8 mg), chlorbutanol (5 mg), excipient	102.3	1.8
Balsoprim (Juste, Madrid)	Per 5 ml suspension: sulfamethoxazole (133 mg), trimethoprim (27 mg), bromhexine chlorhydrate (2.5 mg), sodium saccharin (10 mg), excipient	101.2	1.7
Bronquimucil (Uriach, Barcelona)	Per 100 ml suspension: sulfamethoxazole (4000 mg), brovanexine chlorhydrate (250 mg), trimethoprim (800 mg), sodium saccharin (240 mg), saccharose (50 g), excipient	99.8	1.6
Broncobactifor (Andrómaco, Torrejón de Ardoz, Madrid)	Per 7.5 ml suspension: sulfamethoxazole (400 mg), trimethoprim (80 mg), bromhexine (4 mg), sodium ciclamate (30 mg), saccharin (7.5 mg), excipient	102.8	2.1

Table 10.2 (continued).

Formulation	Composition	Recovery %	RSD (%) n = 5
Brongenit (Elfar-Grag, Fuenlabrada, Madrid)	Per capsule: sulfamethoxazole (400 mg), trimethoprim (80 mg), excipient	103.1	3.2
Broncorema (Septa Farma, Viladecans, Barcelona)	Per 5 ml suspension: sulfamethoxazole (200 mg), trimethoprim (40 mg), guaifenesin (75 mg), sodium saccharin (15 mg), excipient	100.8	2.5
Pulmosterin Duo (Normon, Madrid)	Per 5 ml suspension: sulfamethoxazole (200 mg), trimethoprim (40 mg), bromhexine chlorhydrate (2.5 mg), guaifenesin (25 mg), sodium benzoate (20 mg), sodium saccharin (10.5 mg), saccharose (2.5 mg), excipient	100.2	2.8
Traquivan (Instituto Llorente, Madrid)	Per 5 ml suspension: sulfamethoxazole (133 mg), trimethoprim (27 mg), dihydrocodeine bitartrate (5 mg), sodium saccharin (2.5 mg), excipient	97.3	0.3

hydrochlorothiazide, and the vasodilator hydralazine, are associated, was studied [14]. It was considered that a mobile phase allowing the complete separation of the eight drugs, in an appropriate analysis time, should be useful for the analysis of any pharmaceutical containing two or more of these drugs.

The retention of the drugs was checked to be accurately described by the equation:

$$1/k = c_0 + c_1 [M] + c_2 \varphi + c_3 [M] \varphi \quad (10.1)$$

where $[M]$ is the micelle concentration, and φ the concentration of modifier (v/v). The optimization of the resolution was performed using the normalized product of the overlapped fractions:

$$r = \prod_{i=1}^n \frac{O_{i,i+1}}{\left(\sum_{i=1}^n \frac{O_{i,i+1}}{n} \right)^n} \quad (10.2)$$

being:

$$O_i = 1 - w'_i/w_i \quad (10.3)$$

where w_i is the total area of a given peak, and w'_i the area of the peak overlapped by other peaks. Because of the excessive retention of metoprolol and oxprenolol in mobile phases containing low concentrations of SDS and 1-propanol ($k = 100$ and 178 for metoprolol and oxprenolol, respectively, for a 0.1 M SDS mobile phase without modifier), the optimization study was made within the concentration ranges 0.10 - 0.15 M SDS, and 5 - 15% (v/v) 1-propanol.

The contour map of global resolution depicted in Fig. 10.6 was drawn using the chromatographic data (retention factors, efficiencies and asymmetry factors) of the drugs obtained in five mobile phases (0.1 M SDS-5% 1-propanol, 0.1 M SDS-15% 1-propanol, 0.125 M SDS-10% 1-propanol, 0.15 M SDS-5% 1-propanol, and 0.15 M SDS-15% 1-propanol). It can be observed that the resolution is maximum and scarcely modified over a wide region of the variable space, between 5 and 12% 1-propanol. In this region, the peaks of the eight drugs appeared well resolved. A mobile phase of 0.15 M SDS-7% 1-propanol was selected for the analysis of the pharmaceuticals, since it permitted the determination of the β -blockers, diuretics and hydralazine, within the same run, with analysis times below 20 min. The chromatogram of the mixture of drugs for this mobile phase has already been shown in Chapter 8 (Fig. 8.19). For the analysis of other combinations of these or other drugs, the simple optimization method described will permit the rapid selection of a suitable mobile phase.

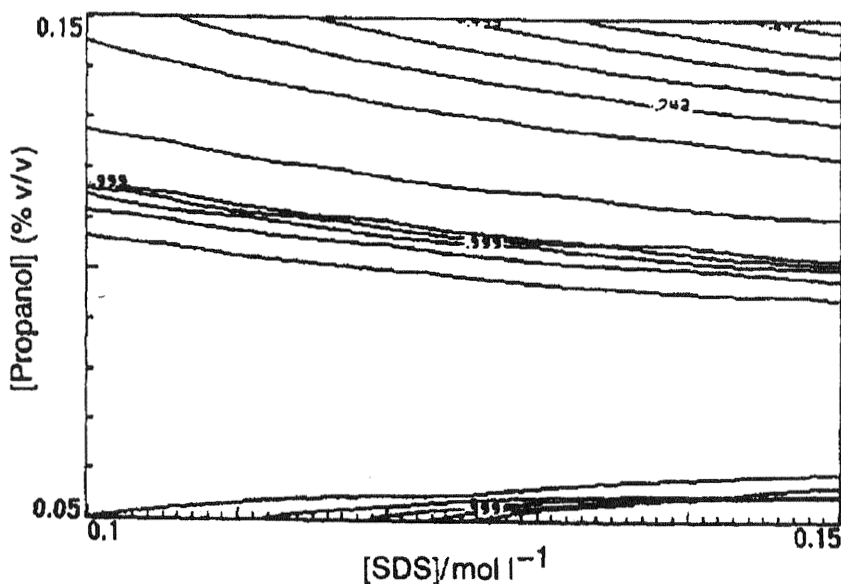


Figure 10.6 Contour map of global resolution for the separation of a mixture of three β -blockers (atenolol, metoprolol and oxprenolol), four diuretics (amiloride, bendroflumethiazide, chlorthalidone and hydrochlorothiazide), and hydralazine, eluted with mobile phases of SDS and propanol at pH 3. Reprinted from Ref. 13 with permission of the Royal Chemical Society.

III.4. *Comparison of Micellar and Conventional Aqueous-Organic Mobile Phases*

Micellar mobile phases are often claimed to be a real alternative, mostly based on their solvent properties and different selectivity. However, in the MLC literature, only a few reports compare the performance of RPLC in the analysis of samples when both types of mobile phases are used. One of these reports deals with the determination of nine β -blockers (atenolol, carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol and timolol) in tablets, capsules and ophthalmic solutions, and represents an example where the MLC technique is really competitive [9].

β -Blockers are used in the treatment of neurological, neuropsychiatric and cardiovascular disorders. The compounds are isoprenaline derivatives and contain an alkanolamine side-chain terminating in a secondary amino group. Their wide range of hydrophobicity makes the RPLC determination of mixtures with aqueous-organic mobile phases difficult. However, interesting results were obtained in MLC. The optimization of the separation of β -blockers showed that a mobile phase of 0.15 M SDS-15% 1-propanol at pH 3, gave a relatively large solvent strength, which permitted the elution of all β -blockers in less than 15 min. The addition of 1-propanol and an acid medium greatly increased the efficiency of the chromatographic peaks.

Aqueous-organic mobile phases of diverse composition have been recommended for the determination of different β -blockers in pharmaceuticals. The chromatographic procedures proposed in the USP-XXII Pharmacopeia [15], British Pharmacopeia [16], and Analytical Profiles of Drug Substances [17], employ aqueous mobile phases containing 35-60% (v/v) acetonitrile or methanol, buffered at pH 3-4 with phosphate. It was checked that 40:60 methanol-0.05 M phosphate at pH 3 eluted most β -blockers in adequate times. Atenolol required however a mobile phase of lower strength, such as 22:78 methanol-0.01 M phosphate (pH 4.3), since its chromatographic peak overlapped with the noise at the head of the chromatogram with the previous eluent. The retention of celiprolol, labetalol, metoprolol, oxprenolol, and propranolol, with the weaker mobile phase was however too high [9].

Table 10.3 Chromatographic Parameters of Several β -Blockers Eluted with SDS-1-Propanol and Methanol-Water Mobile Phases [9].^a

Compound	0.1 M SDS					
	5% 1-Propanol			15% 1-Propanol		
	k	N	B/A	k	N	B/A
Acebutolol	15.6	940	1.9	7.8	1530	1.5
Atenolol	7.3	1640	1.3	3.1	1970	1.3
Carteolol	11.1	1260	1.4	5.0	1870	1.3
Celiprolol	24	510	2.4	10.6	1140	1.8
Labetalol	33	980	1.2	15.8	1640	1.3
Metoprolol	25	1140	1.8	11.2	1880	1.5
Nadolol	12.2	670	1.8	5.4	2220	1.2
Oxprenolol	38	1320	1.7	16.3	1480	1.9
Propranolol	46	1190	1.2	19.1	2110	1.4
Timolol	27	1420	1.6	10.5	2330	1.3

Table 10.3 (continued).

Compound	0.15 M SDS					
	5% 1-Propanol			15% 1-Propanol		
	k	N	B/A	k	N	B/A
Acebutolol	10.5	720	1.9	5.3	1380	1.4
Atenolol	5.0	1360	1.3	2.3	1620	1.3
Carteolol	7.6	780	1.7	3.5	1450	1.3
Celiprolol	16.3	420	2.6	7.2	1100	1.7
Labetalol	21	900	1.2	10.5	1690	1.2
Metoprolol	17.0	990	1.6	7.4	1760	1.4
Nadolol	8.9	850	1.2	3.7	1760	1.2
Oxprenolol	25	1150	1.6	10.7	1700	1.5
Propranolol	29	1120	1.2	12.3	1840	1.4
Timolol	18.0	1080	1.6	7.2	2040	1.3

Table 10.3 (continued).

Compound	Methanol- $\text{NaH}_2\text{PO}_4^b$		
	k	N	B/A
Acebutolol	-	-	-
Atenolol	4.4	70	5.2
Carteolol	1.5	980	1.8
Celiprolol	8.2	80	4.8
Labetalol	8.6	1460	1.7
Metoprolol	5.1	330	3.1
Nadolol	1.8	1180	1.5
Oxprenolol	10.6	130	4.0
Propranolol	22	180	3.8
Timolol	4.1	330	3.2

^a k, retention factor, N, efficiency (plates); B/A, asymmetry factor.

^b A 40:60 methanol-0.05 M NaH_2PO_4 mobile phase at pH 3 was used, except for atenolol for which a 22:78 methanol-0.01 M NaH_2PO_4 mobile phase at pH 4.3 was needed.

The chromatographic parameters (retention factors, efficiencies and asymmetry factors) obtained with the selected SDS micellar and methanol-water mobile phases are shown in Table 10.3. The elution order is the same in both cases, except for celiprolol, metoprolol and timolol, which eluted at close times with the micellar eluents. On the other hand, the peaks obtained with the SDS-propanol eluents had better characteristics than the peaks in the aqueous-organic eluents, with a tenfold increase in efficiency for some β -blockers. The peaks in the aqueous-organic mobile phases were often very asymmetrical. The determination of the whole set of drugs was possible with a unique micellar mobile phase, with retention times of less than 15 min, whereas two different aqueous-organic mobile phases were needed to make the same analyses.

Table 10.4 gives the results of the analysis of pharmaceuticals containing several β -blockers, using micellar and methanol-water mobile phases. In both cases, the pharmaceuticals were dissolved in the solution used as mobile phase. The analyses were made by quintuplicate, and an average value of the measurements was taken to calculate the amount of drug. The recoveries were usually in the 96-103% range for the micellar mobile phase, and in the 89-103% range for the aqueous-organic eluent. The low recovery obtained for some β -blockers with the aqueous-organic mobile phase was probably due to an incomplete extraction of the drug, when the pharmaceutical was dissolved. The 107% recovery achieved with propranolol in Sumial 10 was checked by repeated analyses of the formulation in different days.

III.5. Special Mobile Phases

Table 10.5 shows the experimental conditions (stationary phases, mobile phase compositions and detection wavelengths) and figures of merit (linear ranges and limits of detection) of several chromatographic procedures, that employ micellar mobile phases for the analysis of diverse samples. Some procedures have already been commented on in this chapter. However, some aspects still deserve be reviewed, as the use of special mobile phases containing two surfactants or microemulsions of SDS and pentanol.

Table 10.4 Analysis of Pharmaceuticals Containing Several β -Blockers [9]

Compound	Formulation (laboratory)	Composition (mg)	Found ^a mg	RSD (%) ^a n = 5	Found ^b mg	RSD (%) ^b n = 5
Atenolol	Blokium 50 (Prodes, Sant Just Desvern, Barcelona)	Per tablet: atenolol (50), excipient	49.9	1.4	44.5	2.5
Carteolol	Arteolol (Lácer, Barcelona)	Per tablet: carteolol chlorhydrate (5), lactose and other excipients	4.80	0.6	4.26	0.9
	Elebloc 1% (Cusí, El Masnou, Barcelona)	Per ml: carteolol chlorhydrate (10) in aqueous medium	9.94	0.4	10.6	2.8
	Mikelan 1% (Miquel-Otsuka, Barcelona)	Per ml: carteolol chlorhydrate (10), benzalkonium chlorhydrate (0.05)	9.66	0.8	10.66	0.5
Celiprolol	Cardem (Rhone-Poulenc Rorer, Alcorcón, Madrid)	Per tablet: celiprolol chlorhydrate (200), excipient	197	2.5	214	0.6
Labetalol	Trandate (Glaxo, Aranda de Duero, Burgos)	Per tablet: labetalol chlorhydrate (100), lactose and other excipients	99.0	0.6	97.0	1.0

Table 10.4 (continued).

Compound	Formulation (laboratory)	Composition (mg)	Found ^a mg	RSD (%) ^a n = 5	Found ^b mg	RSD (%) ^b n = 5
Metoprolol	Lopresor (Padró, Barcelona)	Per tablet: metoprolol tartrate (100), excipient	99.4	1.8	89.4	1.9
Nadolol	Solgol 40 (Uriach, Barcelona)	Per tablet: nadolol (40), excipient	40.0	1.8	38.5	0.8
Oxprenolol	Trasicor (Ciba-Geigy, Barcelona)	Per tablet: oxprenolol chlorhydrate (80), excipient	84.5	1.1	77.0	0.5
Propranolol	Sumial 10 (Zeneca Farma, Porriño, Pontevedra)	Per tablet: propranolol chlorhydrate (10), lactose and other excipients	10.7	1.7	10.3	0.8
	Sumial Retard (Zeneca Farma, Porriño, Pontevedra)	Per capsule: propranolol chlorhydrate (160), excipients	165	1.1	151	0.6
Timolol	Blocadrem (Glaxo, Aranda de Duero, Burgos)	Per tablet: labetalol chlorhydrate (100), lactose and other excipients	99.0	0.6	97.0	1.0

^a SDS-1-propanol micellar mobile phase.^b Methanol-water mobile phase.

Table 10.5 Experimental Characteristics of Some MLC Procedures for the Determination of Compounds in Diverse Samples

Compounds	Samples; stationary phases; mobile phase compositions; detection wavelengths; checked linear ranges; limits of detection	Ref.
Fungicides: sodium <i>N</i> -methylthiocarbamate, sodium <i>N,N</i> -dimethylthiocarbamate, ammonium tetramethylenedithiocarbamate, sodium <i>N,N</i> -diethylthiocarbamate, and disodium ethylenebisdithiocarbamate	Pond water; μ -Bondapak CN; 0.0125 M CTAB-30% methanol in 0.018 M phosphate buffer at <i>pH</i> 6.8; 254 nm.	3
Proteins: bovine serum albumin, chymotrypsinogen, β -lactoglobulin, lysozyme, myoglobin, ovalbumin, and thyroglobulin	Mixtures of proteins and beef heart (myoglobin); Supelcosil LC-8; Neodol 91-6 in 0.05 M phosphate buffer at <i>pH</i> 7; 280 nm, fluorescence detection for myoglobin: λ_{exc} = 280 nm, λ_{em} = 320 nm.	2
Vanillin and ethylvanillin	Smoking tobacco; Radicalpak C18; 6% Brij-35; 280 nm.	18
Folylpolyglutamate hydrolase activity	Crude tissue extracts; Whatman PXS 10/25 ODS; 0.2 M SDS; 254 nm.	8
Berberine-type alkaloids: coptisine (I), jatrorrhizine (II), palmatine (III), and berberine (IV)	Coptis rhizome, phellodendron bark and chinese patent medicines; Bondapak Phenyl; 0.12 M SDS, previous extraction with methanol-0.1 M tartaric acid (7:3)-0.12 M SDS; 254 nm; LODs (ng), 12 (I), 7 (II), 11 (III), and 12 (IV).	19
Acetaminophen (I), pseudoephedrine chlorhydrate (II), and chlorpheniramine maleate (III)	Pharmaceuticals (tablets); Zorbax CN; 1.8% Brij 35-0.012 M SDS-4.5% 1-propanol at 65°C; 310 nm (I), 220 (II and III); linearity (mg/ml), 6-21 (I), 0.27-1.36 (II), and 0.019-0.12 (III).	20

Table 10.5 (continued)

Compounds	Samples; stationary phases; mobile phase compositions; detection wavelengths; checked linear ranges; limits of detection	Ref.
Acetylsalicylic acid	Pharmaceuticals (tablets); C18 column; 0.02 M SDS-15% 1-propanol in acetic acid at pH 3; 280 nm.	21
Phenothiazine-type drugs: oxopromethazin, dioxopromethazin, diethazin, promethazin, promazin, chlorphenethazin, chlorpromazin, perphenazin, and fluphenazin	Degradation studies; LiChrosorb C8; 0.005 M CTAB in 0.1 M phosphate buffer at pH 4.6 and 40°C; 254 nm.	22
2-Ethylhexyl-4-dimethylaminobenzoate, 2-ethylhexyl-4-methoxycinnamate, and 2-hydroxy-4-methoxybenzophenone	Sunscreen cosmetic products; Hypersil C8; 0.1 M SDS-10% 2-propanol-0.3% triethylamine at pH 3; 300 nm.	23
Diuretics: acetazolamide (I), amiloride (II), bendroflumethiazide (III), bumetanide (IV), chlorthalidone (V), hydrochlorothiazide (VI), spironolactone (VII), triamterene (VIII), and xipamide (IX)	Pharmaceuticals (tablets and suspensions); Spherisorb ODS; 0.05 M SDS-3% 1-propanol; 220-224 nm, except for III and V (274 nm), and VII and VIII (242 nm); LODs (µg/ml), 0.037 (I), 0.057 (II), 0.019 (III), 0.0032 (IV), 0.0052 (V), 0.0036 (VI), 0.55 (VII), 010 (VIII), and 0.0040 (IX).	24
Diuretics: amiloride, bendroflumethiazide, chlorthalidone, spironolactone, and triamterene	Pharmaceuticals (tablets); Spherisorb ODS; 0.07 M SDS-0.5% 1-pentanol; 224 nm (I), 274 (II and III), and 242 nm (IV and V); LODs, <0.2 µg/ml.	25

Table 10.5 (continued)

Compounds	Samples; stationary phases; mobile phase compositions; detection wavelengths; checked linear ranges; limits of detection	Ref.
Maleic hydrazide	Tobacco; Hypersil ODS; 0.004 M CTAB in 0.04 M phosphate buffer at pH 7; 330 nm; linearity, 29-249 µg/ml; LOD, 20 µg/g tobacco.	26
Sulfonamides: sodium sulfacetamide (I), sulfadiazine (II), sulfaguanidine (III), sulfamerazine (IV), sulfamethizole (V), sulfamethoxazole (VI), sulfanilamide (VII), and sulfathiazole (VIII)	Pharmaceuticals (tablets, pills, capsules, suspensions and drops); Spherisorb ODS-2; 0.05 M SDS-2.4% 1-pentanol, precolumn azo dye derivatization with nitrite and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; 550 nm; LODs (µg/ml), 0.2 (I, IV, V, VIII), 0.02 (II), 0.1 (III, VI), and 0.3 (VII).	11
Catecholamines: L-dopa, 2-methyldopa, epinephrine, dopamine, and isoproterenol	Pharmaceuticals (tablets, capsules, injections and sprays); Spherisorb ODS-2; 0.1 M SDS-5% 1-propanol in phosphate buffer at pH 3; 280 nm; linearity, 8×10^{-6} - 8×10^{-5} M; LODs, 4-7 ng/ml.	27
Human growth hormone	<i>Escherichia coli</i> fermentation broth; Nucleosil C4; 0.035 M SDS-20 to 30% 1-propanol at pH 6.4 and 60°C; 214 nm; linearity, 50-800 µg/ml.	7
Steroids: dydrogesterone (I), medroxyprogesterone (II), medroxyprogesterone acetate (III), nandrolone (IV), nandrolone decanoate (V), progesterone (VI), stanozolol (VII), testosterone enanthate (VIII), and testosterone propionate (IX)	Pharmaceuticals (tablets, injections, suspensions and gels); Spherisorb ODS-2; 0.1 M SDS-7% 1-pentanol; 292 nm (I), 246 nm (II-VI, VIII, IX), and 228 nm (VII); linearity, 0.8-6 µg/ml; LODs (ng/ml), 7.1 (I), 13 (II), 24 (III), 21 (IV), 220 (V), 34 (VI), 860 (VII), 105 (VIII), and 70 (IX).	28

Table 10.5 (continued)

Compounds	Samples; stationary phases; mobile phase compositions; detection wavelengths; checked linear ranges; limits of detection	Ref.
Amino acids: glycine, lysine, threonine, and methionine	Pharmaceuticals (pills, capsules, drops and powders); Spherisorb ODS-2; 0.05 M SDS-3% 1-propanol at pH 3, with precolumn derivatization with <i>o</i> -phthalaldehyde and <i>N</i> -acetyl- <i>L</i> -cysteine; 330 nm; linearity, 4×10^{-5} - 5×10^{-4} M; LODs, 1.3×10^{-7} - 6.5×10^{-7} M.	13
Caffeine	Pharmaceuticals (tablets, capsules and pills); Spherisorb ODS-2; 0.05 M SDS-1.5% 1-propanol; 273 nm.	29
Theophylline	Pharmaceuticals (tablets, capsules and syrups); Spherisorb ODS-2; 0.05 M SDS-3% 1-propanol; 273 nm.	30
β -Blockers: atenolol, metoprolol and oxprenolol; diuretics: amiloride, bendroflumethiazide, chlorthalidone, and hydrochlorothiazide; vasodilator: hydralazine	Pharmaceuticals (tablets and capsules); Spherisorb ODS-2; 0.15 M SDS-7% 1-propanol in 0.01 M phosphate buffer at pH 3; 225 nm; linearity, 0.2-25 μ g/ml.	14
β -Blockers: atenolol, carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol, and timolol	Pharmaceuticals (tablets, capsules and ophthalmic solutions); Spherisorb ODS-2; 0.15 M SDS-15% 1-propanol in 0.01 M phosphate buffer at pH 3; 225 nm.	9
Fungicide: thiram	River water; Spherisorb ODS-2; 0.01 M CTAB-20% acetonitrile in 0.01 M phosphate buffer at pH 6.3; 254 nm; linearity, 0.025-30 μ g/ml; LOD, 25 ng/ml.	31

a) Mobile Phases Containing Two Surfactants

Simultaneous quantitation of the active ingredients commonly used in cold-allergy tablets, acetaminophen, pseudoephedrine and chlorpheniramine, is extremely difficult if not impossible using conventional RPLC. These drugs have vastly different functional groups and polarities. Acetaminophen is a polar derivative of phenol, and can be retained on a reversed-phase column only by using very weak aqueous-organic mobile phases. Pseudoephedrine, a secondary amine derivative of benzyl alcohol is also polar and exhibits chromatographic behavior similar to acetaminophen. In contrast, chlorpheniramine is a nonpolar tertiary amine requiring very strong mobile phases or ion-pairing to elute in a reasonable amount of time. Moreover, acetaminophen is present at much higher levels than either pseudoephedrine or chlorpheniramine, and has a large molar absorptivity. The dilemma in developing a chromatographic procedure for the three compounds is to separate acetaminophen and pseudoephedrine adequately, and still elute chlorpheniramine in a reasonable time. The use of an isocratic mixed micellar mobile phase succeeded in this simultaneous determination [20].

Usually, column choice in MLC parallels column choice in conventional RPLC. In developing the analytical method for the three drugs, C18, C8 and cyano columns were all tried with anionic, cationic and nonionic surfactants, with varying degree of success. Chlorpheniramine could not be eluted from C18 or C8 columns, unless the surfactant concentration was greater than 0.2 M. This system was undesirable since the viscosity of the mobile phase caused excessive backpressure, and acetaminophen and pseudoephedrine were not suitably retained on the column. With lower concentrations of surfactants, it was not possible to separate acetaminophen and pseudoephedrine adequately, and chlorpheniramine was retained too long. Otherwise, when the cyano column was used with a mobile phase of Brij-35, pseudoephedrine eluted in the dead volume. This system was however useful for the separation of acetaminophen and chlorpheniramine. Instead, when mobile phases of SDS were attempted on the cyano column, acetaminophen and pseudoephedrine were adequately separated, but chlorpheniramine was retained too long, resulting in a broad rounded peak.

The comparison of the chromatograms obtained on the cyano column suggested that some combination of the two surfactants could yield the desired separation. Addition of 5 mM SDS to a Brij-35 mobile phase shifted the retention time of pseudoephedrine almost 7 min away from the injection volume. At this concentration, SDS existed as monomers in solution, probably solely functioning as an ion-pairing species. Successive manipulations of the Brij-35/SDS ratio ultimately led to the conditions necessary to separate the three components (Fig. 10.7). 1-Propanol was added for a better control of the separation. The efficiency of the chromatographic peaks was increased maintaining the column at 65°C. Acetaminophen was monitored at 310 nm; approximately 6.5 min after the peak crest time for acetaminophen, the wavelength was automatically changed to 220 nm to detect the peaks for pseudoephedrine and chlorpheniramine.

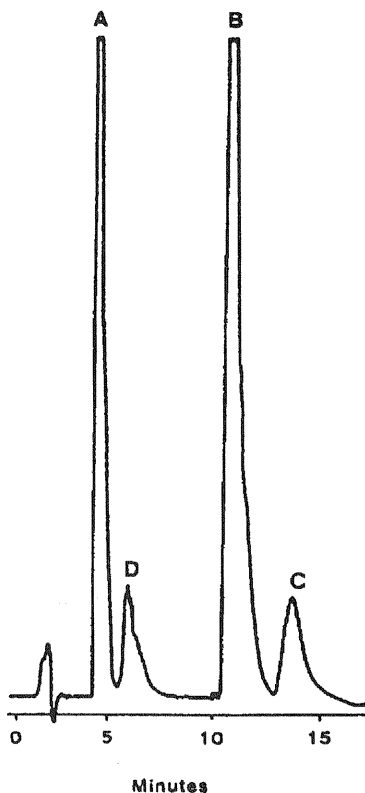


Figure 10.7 Chromatogram of a pharmaceutical preparation: (A) acetaminophen, (B) pseudoephedrine, (C) chlorpheniramine, (D) excipient. Column: cyano at 65°C; mobile phase: 18 g Brij® 35 and 3.46 g SDS dissolved in 1 liter of water containing 4.5% of *n*-propanol. Reprinted from Ref. 20 with permission of Elsevier.

b) Use of a Microemulsion of SDS and 1-Pentanol as Mobile Phase

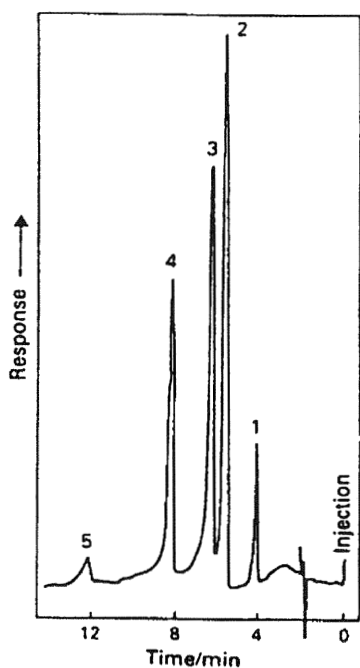
Anabolic steroids stimulate protein synthesis and produce a considerable weight increase. These compounds are used in specific dietary therapies in pathological processes characterized by a negative nitrogen balance, such as some debilitating chronic diseases, during radiotherapy and after major surgery or trauma. The high hydrophobicity of steroids makes them strongly associated with the nonmodified alkyl chains of a C18 stationary phase. The retention of a group of steroids usually administered (dydrogesterone, medroxyprogesterone, medroxy-progesterone acetate, nandrolone, nandrolone decanoate, progesterone, stanozolol, testosterone enanthate, and testosterone propionate) was thus extremely high with a pure micellar mobile phase of SDS. Therefore, the elution strength had to be increased through the addition of 1-pentanol to the mobile phase. However, for low 1-pentanol contents, the retention factors were still excessively high. Only the addition of 7% 1-pentanol to 0.1 M SDS, which created a microemulsion, gave adequate retentions ($k < 14$) and acceptable efficiencies [28]. In a water-pentanol-SDS ternary system, with 7% 1-pentanol, the minimum concentration of SDS required to obtain a microemulsion was 0.065 M. Below this value, a emulsion was obtained. Almost any other compound present in the formulations eluted at the beginning of the chromatograms with a mobile phase of 0.1 M SDS-7% 1-pentanol of high elution strength. The proposed method is therefore strongly group-selective for the steroids, and interesting separations inside the group are also possible.

Many formulations containing steroids are injectable solutions. The oily excipient used in these formulations is an important interference in the analytical procedures reported in the literature, even in those proposed in the Pharmacopeias [15, 16], that utilize spectrophotometry or RPLC with aqueous-organic mobile phases. These procedures require previous separation of the steroids, which frequently leads to low and variable recoveries. With the MLC procedure, the sample preparation step is simple, since the oily media are easily dissolved in the SDS solution and injected directly in the chromatograph.

IV. MISCELLANEOUS APPLICATIONS

IV.1. Pesticides

Micellar chromatography shows promise for the separation of labile dithiocarbamate species with metabolites of toxicological significance, which are commonly employed as pesticides, and hence, may be present in industrial effluents. Conventional methods for the determination of dithiocarbamates are susceptible to interferences and require careful sample clean-up procedures prior to derivatization, to obtain accurate and reproducible results.



A simple MLC procedure was described for the simultaneous determination of five dithiocarbamates (sodium *N*-methyldithiocarbamate, sodium *N,N*-dimethyldithiocarbamate, ammonium tetramethylene-dithiocarbamate, sodium *N,N*-diethyldithiocarbamate and disodium ethylenebisdithiocarbamate) [3], which employed C18 or cyano columns and micellar solutions of CTAB-methanol buffered at pH 6.8 with 0.02 M phosphate. Pond-water samples were used to assess the potential of MLC in the analysis of environmental samples. For the analyses, pond water was filtered and passed through a C18-bonded column to remove aromatic compounds. Resorcinol was added as an internal standard. A typical chromatogram is shown in Fig. 10.8.

Figure 10.8 Chromatogram of dithiocarbamate (DTC) salts (10 $\mu\text{g/ml}$) in spiked pond water: 1. Resorcinol (internal standard), 2. *N*-methyl-DTC, 3. *N,N*-dimethyl-DTC, 4. ammonium tetramethylene-DTC, 5. sodium *N,N*-diethyl-DTC. Column: C₁₈; mobile phase: 10^{-2} M CTAB-70% methanol in phosphate buffer at pH 6.8. Reprinted from Ref. 3 with permission of the Royal Chemical Society.

IV.2. Tobacco

The practical potential of nonionic MLC was demonstrated by the use of micellar solutions of Brij® 35 in the analysis of tobacco [18]. Samples of smoking tobacco were extracted with an aqueous solution of 30% Brij® 35, and an aliquot of the extract was chromatographically separated without further preparation, with a 6% Brij® 35 mobile phase. Comparison with an aromatic aldehyde standard mixture enabled verification of vanillin and ethylvanillin as two of the extract components. Brij® 35 was chosen for this study over other nonionic surfactants (such as Tritons®, Spans®, Igepals® or Tweens®) on the basis of its commercial availability, high purity, low cost, low toxicity, high cloud temperature, and low background absorbance, compared to the other types of surfactants mentioned. Brij® 35 does not possess a strong chromophore and its absorption is minimal.

In another MLC procedure, maleic acid (MH) was determined in crude tobacco and tobacco products with micellar mobile phases of the cationic surfactant CTAB [26]. MH is a synthetic plant growth regulator widely used in tobacco farming as a suckering control agent. The normal practice is to apply MH to the upper half of tobacco plants shortly after topping. The applied MH then gradually translocates to actively growing tissues on the whole plant. The major component in the methanol extract is the β -D-glucoside of MH. The ground tobacco was treated with 12 M HCl to hydrolyze the conjugate form of MH. After sonication in a water bath above 60°C, the yellow-brown sample turned into a dark slurry. After cooling in a cold water bath, the slurry solution was neutralized by slow addition of sodium hydroxide. Although more than 70% of MH was extracted in the first 20 min, a sonication time of 40 min was necessary to obtain a satisfactory recovery.

Fig. 10.9 depicts a representative chromatogram obtained from a tobacco sample containing 80 μ g/ml of MH. The chromatographic separation was performed isocratically from a C18 column with a mobile phase of CTAB at pH 7. The concentration of CTAB was initially 4 mM for the elution of MH and then increased to 7 mM to speed the elution of strongly retained compounds, before changing back to the initial CTAB composition. The run was stopped after 20 min. The change in mobile phase composition was performed without time-consuming column re-

equilibration between injections. This procedure proved to provide good results in the routine determination of MH in a variety of tobacco samples, with average recoveries of 95% and a detection limit of 20 $\mu\text{g/g}$.

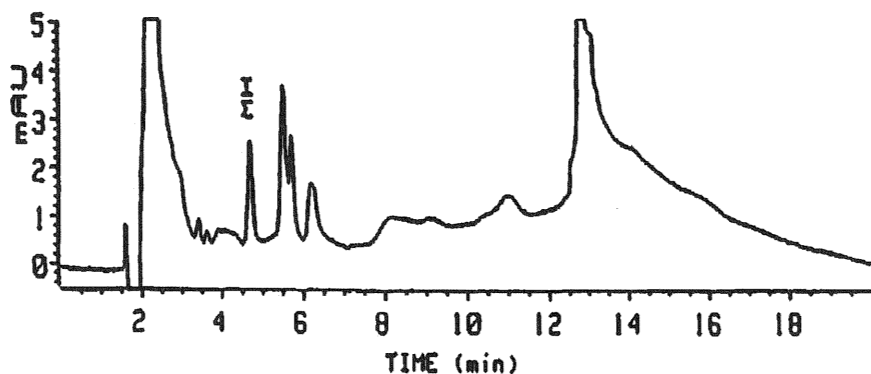


Figure 10.9 Chromatogram of a tobacco sample at a 80 $\mu\text{g/ml}$ level of maleic hydrazide. Column: Hypersil ODS; mobile phase: CTAB in phosphate buffer at pH 7. Reprinted from Ref. 26 with permission of Elsevier.

Adequate buffer strength was important for peak shape and sensitivity. Initially, a buffer of 0.01 M phosphate was used. This buffer gave a reasonable peak shape for a small volume of standard compound, but distorted peaks, and hence, poor limits of detection were observed for tobacco samples. A possible source for this peak distortion could be the presence of a high concentration of NaCl (*e.g.*, approximately 5 M) produced from the acid-base neutralization, which caused slower solute diffusion in the injected solution than in bulk mobile phase. This was further confirmed by obtaining a similarly distorted peak from a standard solution of MH which was 6 M in NaCl. The peak shape of MH in tobacco samples was improved by using a stronger buffer (0.04 M phosphate). This also greatly reduced other possible causes of peak distortion, one of which may be that the pH of the neutralized tobacco solution may not be neutral.

IV.3. Sun Screen Agents

With greater regulation of over-the-counter products by the Food and Drug Administration, a facile analytical method for the determination of sun screen agents in cosmetics was desirable. Determinations based on conventional RPLC have been reported, but due to the complex nature of cosmetics, the preparation of the sample may require an extraction step. A simple chromatographic procedure for the determination of the leading UV absorbers in commercial cosmetic products: 2-ethylhexyl-*p*-dimethylamino-benzoate (PABA), 2-ethylhexyl-*p*-methoxycinnamate (EMC) and 2-hydroxy-4-methoxybenzophenone (oxybenzone) was described. The procedure takes advantage of the easy solubilization of the samples in micellar solution, and employs a micellar mobile phase of SDS-10% 2-propanol with good resolution [23]. The use of external standards minimizes sample preparation even further. The analysis of parabens, utilized as antimicrobial agents in cosmetic preparations, was also demonstrated. The method was applied to a variety of commercial preparations with a sun protection factor ranging from 4 to 15 spf.

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11

Rapid Analysis of Untreated Physiological Fluids

I. INTRODUCTION

The analysis of drugs and their metabolites in physiological fluids is an important issue in pharmaceutical research, clinical chemistry, doping and food quality control and toxicology. Normally, in clinical chemistry, a drug is monitored when any of the following conditions exist: (i) the drug has a narrow therapeutic range, (ii) there is a danger of toxicity, (iii) there is a lack of therapeutic effect, or (iv) there is a forensic need. The therapeutic efficacy of many drugs is closely related to their concentration in blood and tissue, which depends on the dosage, route and frequency of administration. Therapeutic drug monitoring may be necessary to adjust the dose of a drug to the patient's needs. Therefore, appropriate analytical assays that are simple and reliable are desired.

These determinations have been greatly enhanced through the development of High-Performance Liquid Chromatography (HPLC) technology. However, the assay of drugs in physiological fluids presents many problems. Frequently, the drugs are in very low concentration, strongly bound to proteins and in a complex matrix where interference from numerous endogeneous compounds is expected. For this reason, the direct use of Reversed-Phase Liquid Chromatography (RPLC) with aqueous-organic mobile phases, such as mixtures of water with methanol or acetonitrile is usually not feasible.

The high-molecular-mass proteins found in these samples are particularly troublesome. When conventional RPLC silica supports are used, proteins tend to denature and precipitate in the injection valve or at the column head, thus producing obstruction of the interparticulate space and clogging of the system. This leads to a rapid degradation of chromatographic

performance and an increase in back pressure. The most difficult and demanding fluids are those that contain a large fraction of protein, mainly blood, plasma and serum. Cerebrospinal and interstitial fluids, as well as urine, are in general more compatible with liquid chromatographic systems, due to their low protein content.

The harmful proteinaceous material must be removed from the sample prior to injection in an RPLC system, in order to prevent irreversible adsorption to the stationary phase. Several approaches have been used to streamline sample preparation for physiological fluids. One simple approach is to reduce this step to the precipitation of proteins by organics, trichloroacetic acid or sodium hydroxide, or to remove the protein by ultrafiltration. However, very often, the drugs must also be extracted from their matrix and preconcentrated before assay.

Typically, the developed methods include steps such as protein-precipitation, liquid-liquid or solid-phase extraction on disposable cartridges, reextraction, and evaporation, prior to injection into the chromatographic system. Such procedures are time-consuming, require heavy repetitive work, and introduce additional sources of error, since they frequently lead to incomplete recovery of the analytes and dilution. While the use of robotics can allow complex sample preparation to be carried out with high precision and minimal labor cost, the equipment cost and development time of such methods are only justifiable for cases where high sample throughput over an extended time period is expected. An additional problem is the use and disposal of the toxic solvents and chemicals used in the extraction processes. Toxic solvents possess danger not only to the individual working with them but also to the environment. The complexity of these labor-intensive methods have prevented their application for routine clinical use.

Previous sample preparation steps constitute in many applications the main share of the total bioanalytical method. For laboratories with a large number of routine samples for chemical analysis, this means that a lot of time must be devoted to sample work-up procedures. This is largely the reason why much effort has been put into the development of liquid chromatographic systems that can tolerate the direct injection of physiological fluids. Any of the existing HPLC methods performing a sample enrichment and/or a deproteinization step is slower than direct

injection one-step methods, and should preferably include the use of a suitable internal standard to correct for possible errors in the overall procedure and incomplete recovery (even a simple deproteinization step may cause analyte losses due to drug protein binding).

II. THE DIRECT INJECTION APPROACH

II.1. Advantages and Drawbacks

Several authors have intentionally avoided the sample preparation step entirely, by making a direct injection of the physiological sample in the chromatographic system [1]. Minimal sample handling reduces the cost and time of the analyses, increases sample throughput, and decreases error sources owing to minimized risks of losses and chemical changes in the analytes, at the reduced number of steps. This improves the limits of detection (LODs), and the accuracy and precision of the determinations. Another advantage offered is the low sample demand of only a few microliters. For blood analysis, for instance, fingerpuncture samples are adequate for analysis. This is an important consideration in pediatric applications.

There are, however, also occasions where direct injection principles are unsuitable. When the analytes are chemically or biochemically transformed in the physiological fluid, it may be necessary to rapidly transfer them to a nonbiological environment by a liquid-liquid or liquid-solid distribution. Obviously, if only a single specimen has to be analyzed, it can be directly injected into a suitable chromatographic system; the problem arises when there is a series of samples with unstable compounds. In some cases, it may still be possible to stabilize the analytes by the addition of antioxidants and/or enzyme inhibitors.

Direct injection is also limited by the fact that there is no elimination of interferences of both exogeneous and endogeneous compounds, and there is no preconcentration of the drug. Thus, frequently, large volumes of untreated physiological fluids need to be injected, in order to determine drugs

whose therapeutic ranges lie in the ng/mL range. Injection of large volumes creates the further problem of blockage of the analytical column and build-up of strongly retained endogeneous compounds. Also, because there is no sample clean-up, the physiological matrix will produce a large signal eclipsing the peaks of early eluting compounds. Optimization should be achieved by judicious selection of the detection scheme and detector wavelength.

II.2. Direct Injection Techniques

The past decade has witnessed a proliferation of methods and media for direct injection of physiological fluids into HPLC systems. The numerous studies and applications of the principle of direct injection clearly demonstrate that the technique now has matured to a stage where it can be used routinely in bioanalytical laboratories, for the automated analysis of a large number of samples [1].

Conventional RPLC media are not adequate to tolerate direct injection of small analytes in protein-containing matrices. Therefore, special column packings have been designed to minimize the deleterious effects of the adsorption of proteins. Most systems utilize a precolumn for the reception of the physiological fluids, trace enrichment and preliminary clean-up, before the analyte fraction is transferred to the analytical column. The precolumn is changed regularly to keep the system backpressure at normal levels. The most common solid phase for the precolumn is silanized silica, but many other types have been used. The stability index used in these studies is taken as the number of injections (corresponding to a certain total volume of physiological fluid) that reduces the chromatographic efficiency or, alternatively, increases the pressure by a given percentage. Systems which can handle the injection of 25 mL total physiological sample volume, without the need to exchange the precolumn, have been reported.

An important feature of direct injection is that because the same precolumn can be used for all assays in a series of specimens, the risk of nonreproducibility of off-line procedures where a new precolumn is used for each sample, is eliminated. Great care should be taken in order to keep the endogeneous proteins solubilized during chromatography. Certain eluent

restrictions are associated with the use of precolumns to ensure that the matrix components remain in solution (*e.g.*, the concentration of the organic modifier should be kept as low as possible, preferably below 10%).

The large differences in molecular magnitudes between drugs and macromolecules have been exploited. One interesting approach is the pretreatment of small pore silica with plasma [2] and/or solutions containing bovine serum albumin (BSA) [3]. The pores are so small that the proteins do not penetrate into the support, and the macromolecules are only adsorbed onto the external surface of the particles, leaving the largest part of the surface area unaltered. Another concept is the use of a solid phase prepared by attaching a hydrophobic tripeptide to glycerylpropyl-derivatized small pore silica [4]. This packing, named internal surface reversed-phase (ISRP), exposes internal and external surfaces of the support: the former permits hydrophobic partitioning and the latter is a hydrophilic phase, nonadsorptive toward protein.

The precolumn serves the dual function of acting as a guard column, as well as effecting a preseparation of the analytes from the physiological fluid matrix. It can be used in two different ways: in the foreflush or backflush mode. Figure 11.1 shows two common valve configurations [5]. In the first configuration (A), the extraction column is between the injector and the column switching valve and is always operated in the foreflush mode. Solvent delivery to the extraction column is performed by pump 1 which pushes the matrix proteins to the waste. During drug transfer from the extraction column to the analytical column, pump 1 delivers the wash solvent to both columns connected in series. Next the proteins are washed off the extraction column and pump 2 separates the analytes in the analytical column. In the second configuration (B), the extraction column is set across the switching valve. The initial position operates similarly to configuration A, but when the valve is actuated, solvent delivery to both columns in series is performed by pump 2. As in configuration A, the extraction column is forward flushed for elution of the drug.

Another configuration can be derived from B. Again the extraction column is set across the switching valve. At this valve, the line from the analytical pump (pump 2) and the line to the analytical column have their positions switched. Similar to configuration B, when the valve is

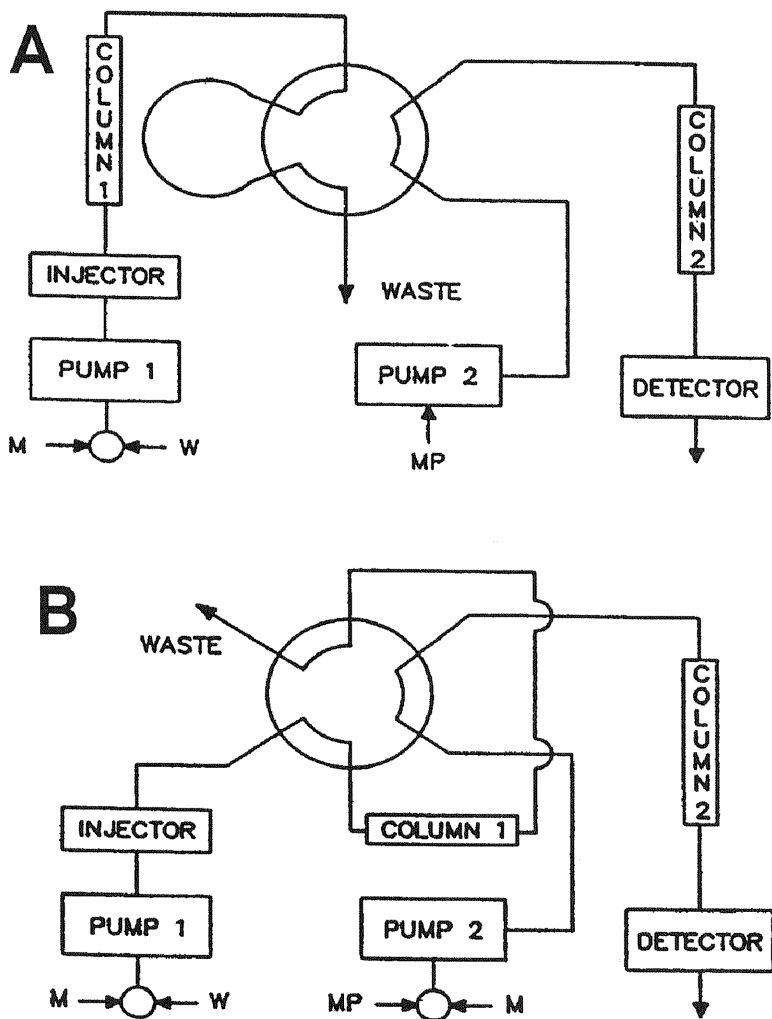


Figure 11.1 Alternative valve configurations: loading pump (pump 1), analytical pump (pump 2), extraction column (column 1), analytical column (column 2), extraction solvent (M), extraction column wash solvent (W), analytical mobile phase (MP). Reversing the Pump2 and Column 2 connections will reverse the flow path in Column 1 during the drug transfer step, allowing solute enrichment at the head of Column 1. Reprinted from Ref. 5 with permission of the American Chemical Society.

actuated, solvent delivery to both columns is provided by pump 2. However, the flow path along the extraction column is reversed thereby backflushing the extraction column to elute the component of interest. In other words, the analytes are not transferred to the analytical column passing all through the extraction column. On the contrary, since they are still retained at the head of this column, they are backwashed. In this way, the analytes are enriched on the top of the precolumn. The system can also consist of two alternating precolumns, which provides an efficient and time-saving operation of the analytical column. The sample is injected and loaded on one of the precolumns and after a purge phase, the analytes are backflushed on the analytical column. During the purge phase a new sample is injected, and while the first sample is eluted on the analytical column, the next sample is backflushed onto the analytical column from the second precolumn [6]. With these techniques, the lifetime of the analytical column is so high that several hundred injections in the low μl range can be performed, without loss of stability. Columns last longer by using small injection volumes, large packing volumes and large total volume of mobile phase through the column.

An alternative of direct injection can be found in the use of conventional RPLC columns and eluents capable of solubilizing the proteins in the physiological matrix, such as triethylammonium acetate buffers with silica packings, or as explained next, solutions of surfactants at a concentration above the critical micellar concentration (cmc). This approach can be a good choice for direct injection.

III. USE OF MICELLAR MOBILE PHASES

III.1. Attractive Advantages

One of the main appeals of Micellar Liquid Chromatography (MLC) is, in fact, the possibility of determining drugs in physiological fluids, without the need of previous separation of the proteins present in the samples. This technique has proved to be the most promising and useful, in order to render a lifetime as long as possible to the chromatographic column, when direct

injection is used. The compatibility with conventional RPLC column packings is particularly attractive.

Micelles tend to bind proteins competitively [7], thereby releasing protein-bound drugs. Therefore, these are free to partition into the stationary phase, whereas the proteins rather than precipitating on the column, are solubilized and swept away harmlessly, eluting with or shortly after the solvent front. Another advantage is that surfactants are nontoxic, nonflammable and relatively inexpensive, in comparison to aqueous-organic solvents. The use of surfactants in direct injection is also much less complex than column-switching procedures which require additional instrumentation (precolumns, switching valves and HPLC pumps), and accurate and precise timing of valve switching for a successful separation.

In MLC, untreated physiological fluids directly injected into the HPLC system possess no difficulty, allowing hundreds of repetitive serial injections with no increase in system pressure, no noticeable clogging of the injection port or analytical column, no changes in retention factors, or system contamination evident. However, it should be reminded that the analytical column should be protected with a guard column to saturate the micellar mobile phase with silica. This guard column should be controlled and replaced after repetitive injections of the physiological samples. It should be unnecessary to comment that samples must be filtered previously to injection to eliminate any particulate matter. Also, it is convenient to inject daily a probe solute, in order to check for possible changes in retention. The pressure of the system should also be monitored to evaluate the precipitation of proteinaceous material into the column.

III.2. Useful Surfactants

MLC has been applied extensively and seems to be rather useful and valid for different solid phases and micellar eluents. The anionic surfactant sodium dodecyl sulfate (SDS) is the most commonly used for direct injection of physiological fluids, but the nonionic surfactant polyoxyethylene(23) dodecyl ether (Brij® 35) has also been employed successfully. Cationic surfactants cause protein to precipitate and cannot be used.

Attempts have been made to use other surfactants, such as sodium decyl sulfate and sodium pentadecyl sulfate [8]. However, it was observed that none of these are able to quantitatively elute BSA when methanol is present in the eluents. In fact, sodium pentadecyl sulfate has limited solubility in eluents containing less than 30% methanol (v/v concentrations of organic solvents are given). One nondenaturing anionic surfactant, sodium desoxycholate was found to elute BSA from model serum injections. A zwitterionic surfactant known as C12 DAPS (3-(dimethyldodecylammonium propanesulfonate) has also been successful in drug analysis [9].

III.3. Mobile Phase Composition

It is necessary to avoid conditions that are unfavorable for micelle formation or for protein solubilization. Grohs et al. [8] studied the range of surfactant and organic solvent compositions which will permit direct injection of physiological samples onto C18 chromatographic columns, and indicated that the eluents typically used in MLC may only represent a subset of the surfactant-containing eluents that will permit direct injection. The authors demonstrated by using a model serum of 0.05 M BSA and a mobile phase of 0.001 M SDS-15% methanol (the cmc of SDS was not reached), that good results and no column clogging occurred after a hundred injections. However, some limitations on the use of organic solvents as additives were found. These limitations are not based on considerations of micelle stability, but rather upon the need to favor surfactant-protein, as well as surfactant-packing interactions. SDS interactions with both protein and C18-bonded phase appears to be too limited in the presence of relatively high amounts of methanol, to allow direct injection of physiological fluids. It is even more problematic with other alcohol modifiers.

Several authors have reported problems of pressure increases and irreproducible retention times, when low concentrations of surfactants are employed (below 0.05 M). Sentell et al. [10] used a micellar mobile phase of 0.02 M SDS-10% 1-propanol in phosphate buffer at pH 3.5, for the determination of the diuretic bumetanide. After several injections of spiked serum, the operating pressure increased noticeably and the retention time of the drug decreased. Using a similar mobile phase, Ameer and Burlingame [11] also observed that serial injections of plasma containing bumetanide

brought about shortening retention times of the analyte. This was explained as changes in the stationary phase characteristics produced by the complex plasma matrix. The determination of bumetanide was appreciably improved by increasing the concentration of SDS to 0.1 M. The chromatograms had much less noisy background, probably due to a better solubilization of the proteins. Finally, Dadgar and Kelly [12] attempted direct injection of plasma fortified with chlorthalidone and furosemide with a mobile phase of 0.05 M SDS and 5% 1-propanol, but a rapid increase in column backpressure occurred, as well as loss of analyte sensitivity.

One reason to limit the amount of organic solvent in eluents designed for direct injection might be the need to maintain an adequate coating of surfactant on the reversed-phase packing, and thereby prevent protein adsorption (see Chapter 4). Protein material can only be effectively removed from the samples and the column packing material, if a sufficiently high surfactant concentration is used in the mobile phase. For this reason, the concentration of the surfactant should be maintained well above the cmc, and the organic solvent content relatively low. The role and integrity of the micelles in the mobile phase is not clear at a low concentration of SDS (*e.g.*, 0.02 M) and high concentration of alcohol (*e.g.*, 10% propanol).

The type of surfactant and modifier, their concentrations, and the pH of the mobile phase have been identified as the key parameters that can be varied to obtain the required resolution between a drug and the components of a physiological sample.

III.4. Displacement of Drug Bound to Proteins

In most cases, the drug bound to proteins is displaced by the surfactant monomers and/or micelles, being completely released for partitioning to the stationary phase. Drug recovery in MLC with direct injection could be however incomplete, and doubled peaks can appear ascribed to the protein-bound and unbound drug (this depends on the nature of the drug, protein binding and mobile phase composition). Examples of this situation have been given for the quantitation of cephalosporins [13,14] and methotrexate [15].

When Haginaka et al. [13] applied the direct injection approach to the determination of cephalosporins, using a C18 column and an SDS eluent, they found that the compounds were not always observed as single peaks, which depended on the pH of the eluent. Fig. 11.2 shows the chromatograms of a mixture of cefmenoxime hemihydrochloride (CMX) and cefotiam dihydrochloride (CTM), eluted with a 0.08 M SDS-8% 2-propanol mobile phase in phosphate buffer at pH 3.3. CMX in serum showed two peaks at retention times of 3.84 and 5.25 min, while CTM gave a single peak. The authors claimed that, as the protein binding of these drugs is approximately 80% and 7-8%, respectively, the two peaks of CMX might be due to drug bound (the peak at a shorter retention time) and not bound (the peak at a longer retention time) to serum proteins. Acidification of the serum sample

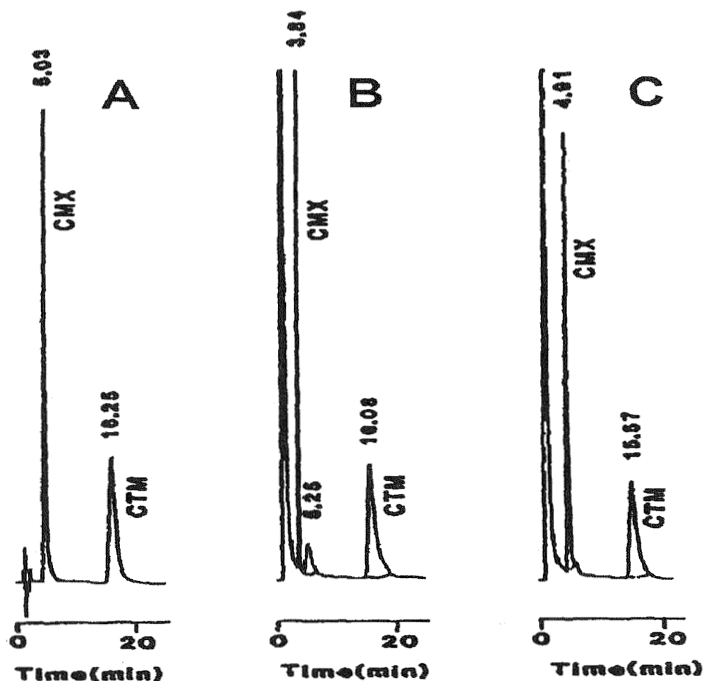


Figure 11.2 Chromatograms of cefmenoxime hemihydrochloride (CMX) and cefotiam dihydrochloride (CTM) in: (A) distilled water, (B) serum sample, and (C) acidified serum sample. Mobile phase: 0.08 M SDS-8% 2-propanol in 0.047 M phosphate buffer at pH 3.3. The figures over the peaks represent the retention times. Reprinted from Ref. 13 with permission of the American Chemical Society.

(pH 1.2) changed the two peaks of CMX into a single peak, as shown in the figure. Also, after ultrafiltration of the serum samples, the peak at longer retention time was only observed in all eluents used. On the assumption that the two peaks of CMX were due to bound and unbound drug, the protein binding estimated as the peak area ratio of the bound to total CMX, was 79%. This suggested that the protein binding of a drug might be determined by using MLC.

For other drugs, or for cephalosporins and methotrexate in other conditions, the recoveries from physiological samples were often close to 100%, which indicated that the protein-bound drug was completely displaced by the micellar phase. These examples also show that, for a strongly bound drug, the alteration of drug-protein binding by changing the conditions, such as pH, is required for the recovery and quantitation of the total drug.

IV. BACKGROUND SIGNAL OF THE MATRIX FLUID

IV.1. Drug Overlapping

A limitation of the direct injection method with micellar mobile phases is the high background signal of the matrix at the beginning of the chromatogram, which may completely overlap the peaks of the analytes, resulting in a useless zone (Fig. 11.3) [16]. The profile of the background depends on the composition of the mobile phase and is due to the presence of proteins and endogeneous compounds. The protein-surfactant complexes, excluded from the pores of the stationary phase support, appear as a broad band at the solvent front and an unidentified endogeneous compound produces a prominent peak that stands out among other smaller peaks. Both may represent occasionally serious interference, but can be reduced at an increased detection wavelength. The background signal of the matrix is also observed in conventional RPLC, even with previous separation steps [17].

It may be convenient to decrease the retention of the protein band and the prominent peak, as much as possible, for the determination of many compounds. Only if the elution of the drug is produced at the end of the

protein band, will the determination be possible. Drugs eluting at shorter times will need extraction procedures.

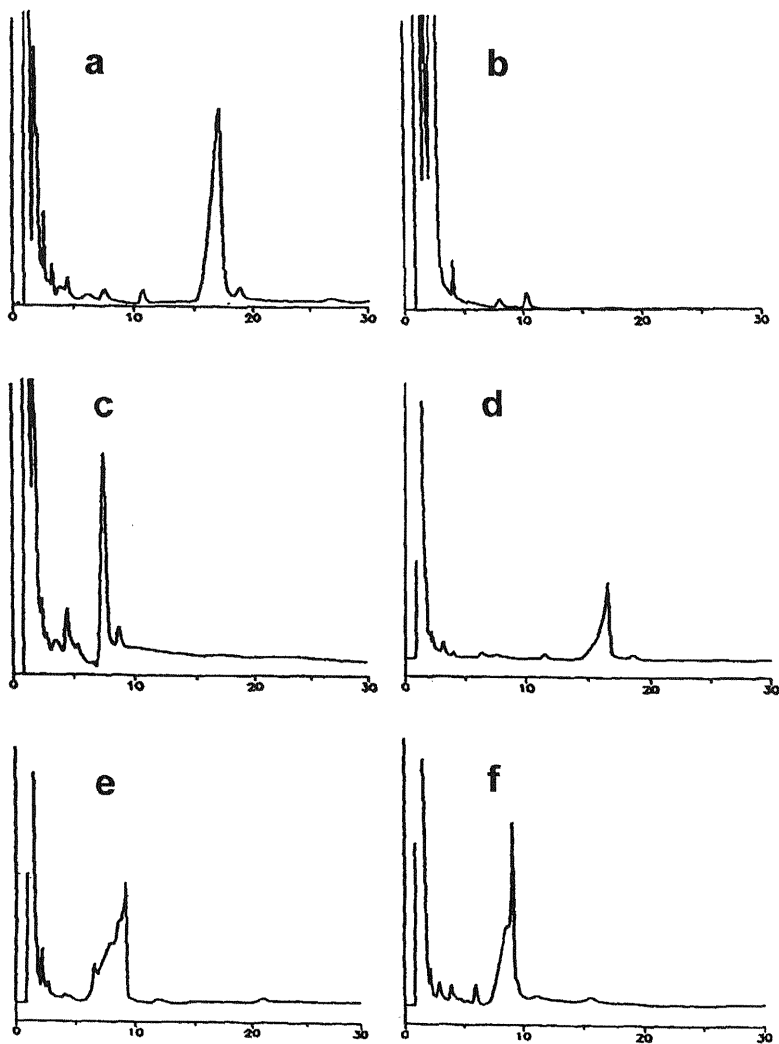


Figure 11.3 Chromatograms of the background of urine matrix eluted with different mobile phases [pH was 4.5 in all cases except for (b) where it was 6.9]: (a) and (b) 0.05 M SDS, (c) 0.15 M SDS, (d) 0.03 M SDS-2% 1-propanol, (e) 0.05 M SDS-4% 1-propanol, (f) 0.07 M SDS-2% 1-propanol. Reprinted from Ref. 16 with permission of the Sociedad Española de Química Analítica and Elsevier.

IV.2. Influence of Mobile Phase Composition

Cline-Love and Fett [18] studied the effect of pH and concentration of surfactant (SDS and Brij® 35) on the retention of the prominent peak, in the chromatogram of urine matrix. These authors observed that the retention of this peak was minimized and remained constant in the pH-range 5.5-7.5, particularly with SDS mobile phases, for all surfactant concentrations studied. At pH ≤ 5.5 , an important increase in the retention of the endogeneous compound was observed for both surfactants. The shape of the k vs. pH curve of the endogeneous compound is sigmoidal, which indicates that it is protonated in acid solution, with a logarithm of the protonation constant, $\log K_H = 4.5-5.0$ (Fig. 11.4) [16]. The retention is also much longer at a decreasing concentration of surfactant (Fig. 11.5). With pure micellar mobile phases, the pH should be in the range of 6-7 in order to minimize the retention of the matrix, or at a lower pH, a larger concentration of SDS should be used. However, the elution of the drugs should also be considered. The retention of many acidic compounds increases at lower pH, whereas at larger SDS concentration, the retention is decreased and the efficiency of the chromatographic peaks deteriorates appreciably.

An alternative of reducing the retention of the matrix is the addition of an alcohol to solutions having moderate concentration of surfactant. In fact, most procedures developed for the determination of drugs by MLC utilize SDS as surfactant together with an alcohol as modifier, due to the excessive retention with pure micellar eluents. A study was made on the variation of the background of physiological matrix (urine) when mobile phases with several modifiers were used [16]. The retention of the prominent peak in the matrix decreased, as usual, with increasing concentration of methanol, 1-propanol and 1-pentanol, following the elution strength of these alcohols. A smaller effect was observed with the protein band.

All these studies showed that the retention of the endogeneous compound giving the prominent peak leaves a detection window for the drugs and is reproducible. The mean retention time of the prominent peak with a mobile phase of 0.042 M SDS-4% 1-propanol at pH 4.5, for samples taken from eleven healthy males and females, was measured to be 8.3 ± 0.3 min (relative standard deviation, RSD = 4%). On the other hand, the RSD for

the position of this peak for 12-16 urine samples of two volunteers, taken along 36 h, was 3-4%.

Figure 11.4 Influence of pH and concentration of SDS on the retention of the prominent peak in the chromatogram of urine matrix. Mobile phases: (1) 0.05 M SDS, (2) 0.1 M SDS, (3) 0.15 M SDS. Reprinted from Ref. 16 with permission of the Sociedad Española de Química Analítica and Elsevier.

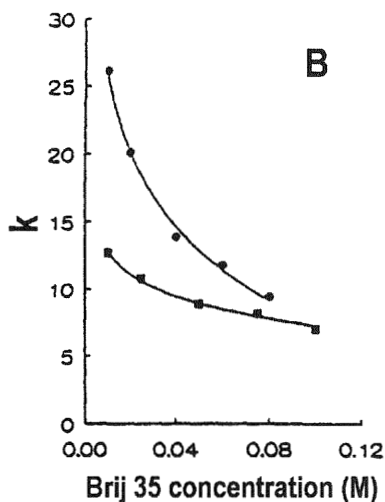
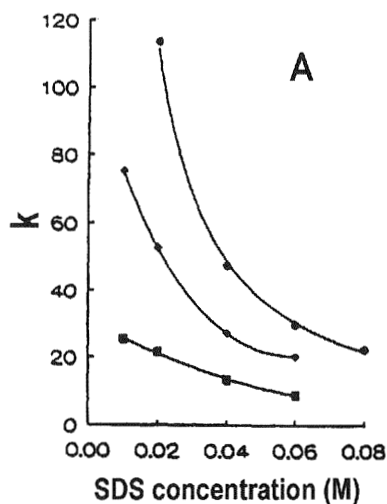
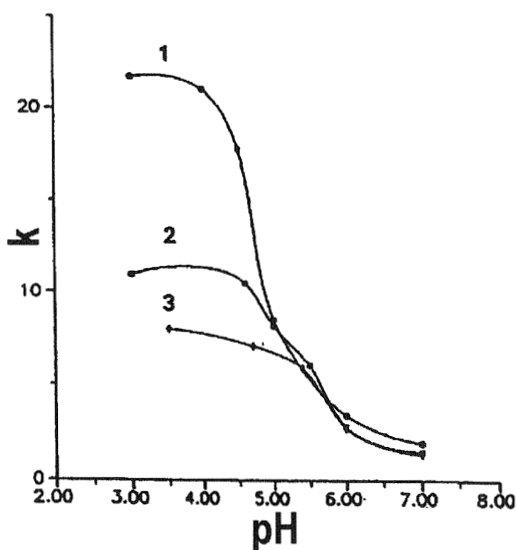


Figure 11.5 Change in the retention factor of the prominent peak in urine matrix, as a function of mobile phase surfactant concentration at various pH: (A) SDS mobile phases at pH: 3.0 (●), 5.0 (◆) and 7.5 (■); and (B) Brij-35 mobile phases at pH: 3.0 (●) and 7.5 (■). Reprinted from Ref. 18 with permission of Elsevier.

The optimization of mobile phase composition should take into account not only the retention of the compound to be analyzed, but also the retention of the matrix. The determination of anticancer 6-thiopurine drugs and their metabolites in untreated serum is a useful example (Fig. 11.6) [19]. With a mobile phase of 0.04 M SDS in 0.01 M phosphate buffer at pH 2.2, the blank serum produced a background response that had completely eluted after 6 min, with the exception of a peak at 8 min. The peaks of 6-thioguanidine riboside and 6-thioguanine were well resolved, but unfortunately, the three earlier-eluted compounds (6-mercaptopurine riboside, 6-thioxanthine and 6-mercaptopurine) were overlapped by the matrix peaks. A lower pH (2.0) permitted complete separation of 6-mercaptopurine from the serum background signal. Under these conditions, 6-thioguanine eluted too late (at around 40 min).

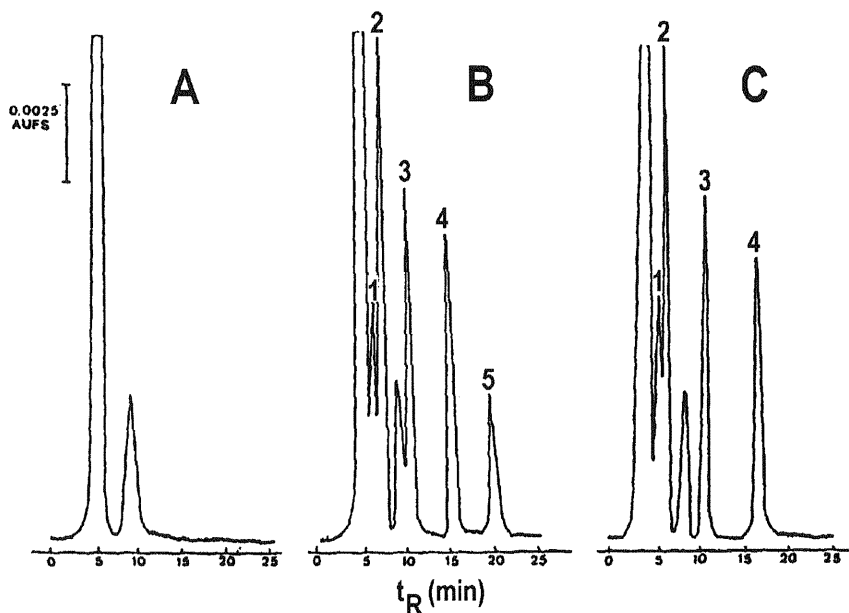


Figure 11.6 (A) Chromatograms of serum blank and (B) and (C) serum spiked with 1 $\mu\text{g/mL}$ of 6-thiopurine compounds. C18 column and mobile phase of 0.04 M SDS in 0.01 M phosphate buffer at pH 2.3 for (A) and (B) and pH 2.0 for (C). Compounds: (1) 6-mercaptopurine riboside, (2) 6-thioxanthine, (3) 6-mercaptopurine, (4) 6-thioguanidine riboside and (5) 6-thioguanine. Reprinted from Ref. 19 with permission of Elsevier.

V. APPLICATION DEVELOPMENT

V.1. The State of the Art

It has been demonstrated that MLC is a reliable chromatographic method, which can greatly improve both the speed and simplicity of the analysis for compounds of interest in physiological fluids. The versatility of this technique encompasses the wide range of drug classes normally monitored, such as analgesics, anticancer drugs, antidepressants, antiepileptics, bactericides, β -blockers, bronchodilators, catecholamines and diuretics, among others. Some of the reasons that explain the few applications reported up-to-date for this technique may be that most of the present extraction/reconstitution methods are well established, and/or the method development in MLC is unfamiliar. The ability to inject physiological samples directly into an MLC system will no doubt in the future be exploited for clinical applications.

The experimental characteristics of some reported chromatographic procedures, that employ micellar mobile phases for the determination of drugs in physiological fluids, are given in Table 11.1. In these procedures, UV-visible detection was used. Other procedures using other detection modes will be commented on in Chapter 12. Spiked samples were usually employed for the development of the procedures. However, an appropriate study of the possibilities of the technique should include the use of metabolites and urinary excretion controls. The physiological samples should be collected at several time intervals post-dose, frozen immediately and stored in the dark, until being analyzed. The stability of the drugs in the samples should also be controlled. Comparison of the samples with a blank serum is always necessary.

V.2. Optimization Protocol

Mobile phase parameters should be properly chosen to improve the selectivity of the separation between a drug and the endogeneous components in the physiological fluids. Optimal control of mobile phase composition, including pH and type and concentration of surfactant and modifier, permits the development of MLC procedures for the determination of virtually any drug in these samples.

Table 11.1 Experimental Characteristics of Some MLC Procedures for the Determination of Drugs in Physiological Fluids with UV-Visible Detection

Compounds	Samples; Stationary Phases; Mobile Phase Compositions; Detection Wavelengths; Checked Linear Ranges; Limits of Detection	Ref.
Acetylsalicylic acid (I), chloramphenicol (II), theophylline (III), acetaminophen (IV), carbamazepine (V), phenobarbitone (VI), phenytoin (VII) and procainamide (VIII)	Serum; Supelcosil LC-18 (I, II), μ -Bondapak C18 (III) and Supelcosil LC-CN (IV, V, VI, VII, VIII); 0.02 to 0.10 M SDS, pH 3 (I); 254 nm; LODs, 0.2-3 μ g/mL.	20
Carbamazepine (I) and theophylline (II)	Serum; Supelcosil LC-CN (I) and Supelcosil LC-18 (II); 0.02 M SDS (I) and 0.05 M SDS (II); 254 nm; linearity (μ g/mL), 10-20 (I) and 2-5 (II).	21
Cephalosporins: cefmenoxime hemihydrochloride and cefotiam dihydrochloride	Serum; Nucleosil C18, 0.08 M SDS-8% 2-propanol in 0.05 M phosphate buffer at pH 3; 260 nm; linearity, 5-100 μ g/mL; LODs, 2 μ g/mL.	13
Cephalosporins: cephalexin (I), cefradine (II), cefotaxime (III) and cefmenoxime (IV)	Serum; Develosil ODS; 0.02 M SDS in 0.05 M phosphate buffer at pH 6.1 (I, II) and 0.15 M in 0.05 M phosphate buffer at pH 3.1 (III, IV) and 40°C; 254 nm.	14
Anticancer drugs: 6-mercaptopurine (I), 6-thioguanine (II) and their metabolites 6-mercaptopurine riboside (III), 6-thioguanine riboside (IV) and 6-thioxanthine (V)	Serum; LiChrosorb RP-18; 0.08 M SDS in 0.01 M phosphate buffer at pH 3 and 30°C; 320 nm; linearity, up to 4 μ g/mL; LODs (μ g/mL), 0.56 (I), 0.21 (II) and 0.10 (IV).	19
Diuretic: chlorthalidone	Plasma; C8 column; 0.05 M SDS-5% 1-propanol in phosphate buffer at pH 5.8, previous extraction with 2-propanol-ethyl ether (1:19), xipamide as internal standard; 235 nm; linearity, 50-800 ng/mL.	12
Diuretic: bumetanide	Serum and urine; Nucleosil RP-18; 0.10 M SDS-3% 1-propanol in 0.05 M phosphate buffer at pH 3.5; 305 nm.	10

Table 11.1 (continued)

Compounds	Samples; Stationary Phases; Mobile Phase Compositions; Detection Wavelengths; Checked Linear Ranges; Limits of Detection	Ref.
Diuretic: hydrochlorothiazide	Urine; Hypersil C18; 0.02 M Brij 35-0.004 M SDS in 0.01 M phosphate buffer at pH 6.5; 271 nm; linearity, 0.3-110 µg/mL.	18
Diuretics: bendroflumethiazide and chlorthalidone	Urine; Spherisorb ODS-2; 0.05 M SDS-5% methanol at 50°C; 224 nm; LODs, 0.1-0.5 µg/mL.	22
Diuretics: amiloride (I), bendroflumethiazide (II), bumetanide (III), chlorthalidone (IV), ethacrynic acid (V), furosemide (VI), spironolactone (VII), triamterene (VIII) and xipamide (IX); also probenecid (X)	Urine; Spherisorb ODS-2; 0.042 M SDS-4% 1-propanol in 0.01 M phosphate buffer at pH 4.5; 254 nm; linearity, 2-15 µg/mL; LODs (µg/mL), 0.8 (I, V), 0.4 (III, VI, VII), 0.3 (IV), 0.2 (VIII, X) and 0.08 (IX).	23
Thiazide diuretics: althiazide (I), bendroflumethiazide (II), chlorothiazide (III), hydrochlorothiazide (IV), hydroflumethiazide (V) and trichlorothiazide (VI); also furosemide (VII)	Urine; Spherisorb ODS-2; 0.05 M SDS-8% 1-propanol, with precolumn azo dye derivatization of the hydrolized diuretics with nitrite and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; 525-550 nm; LODs (µg/mL), 2.9 (I), 2.5 (II), 2.2 (IV), 2.0 (VI) and 1.7 (VII).	24
Anticancer drug: methotrexate	Serum and urine; LiChrospher 100 RP-18; 0.1 M SDS in 0.05 M phosphate buffer at pH 5.7 for serum, pH 5.2 for urine; 305 nm; linearity 0.1-100 µM; LOD 90 nM.	15
Amino acid: proline	Urine; C18; 0.03 M SDS-8% 1-propanol in 0.01 M acetate buffer at pH 5.3 and 40°C, precolum derivatization with 0.001 M Cu ²⁺ ; linearity, up to 300 µg/mL; LOD, 10 µg/mL.	25

Table 11.1 (continued)

Compounds	Samples; Stationary Phases; Mobile Phase Compositions; Detection Wavelengths; Checked Linear Ranges; Limits of Detection	Ref.
Illegal drugs in sport: amiphenazole (I), amiloride (II), amphetamine (III), clostebol (IV), ephedrine (V), phenylpropanolamine (VI), methandienone (VII), methoxyphenamine (VIII), nandrolone (IX) and spironolactone (X)	Urine; Spheri-5 RP-18; 0.1 M SDS-3% 1-pentanol; 260 nm; LODs ($\mu\text{g/mL}$), 2.6 (I), 11 (II), 4.1 (III), 1.2 (IV), 4.2 (V), 2.2 (VI), 0.4 (VII), 8.7 (VIII), 0.07 (IX) and 1.6 (X).	26
Antipyrine metabolites: 4-aminoantipyrine, 4-methylaminoantipyrine and 4-formylaminoantipyrine	Plasma; Nucleosil C18; 0.1 M SDS-2.5% 1-pentanol, extraction with sodium hydroxide and methylene chloride, evaporation to dryness and reconstitution with mobile phase, furosemide as internal standard; 262 nm; linearity, up to 14-18 $\mu\text{g/mL}$; LODs, 10-17 pg/mL .	27
Antipyrine metabolites: 4-aminoantipyrine (I), 4-methylaminoantipyrine (II), and 4-formylaminoantipyrine (III)	Plasma; Spheri RP-18; 0.1 M SDS-2.5% 1-pentanol, C18 solid-phase extraction with methanol and water as eluents; 262 nm; linearity, 2.4-4 $\mu\text{g/mL}$; LODs (ng/mL), 11.5 (I), 17 (II) and 10.5 (III).	28
Bronchodilator: theophylline	Serum; μ -Bondapak phenyl; 0.001 M 3-(dimethyldodecylammonium) propanesulfonate (zwitterionic surfactant)-3% 1-propanol; 273 nm; linearity, 0.5-20 $\mu\text{g/mL}$.	9
Stimulant: caffeine (I) and their metabolites, theophylline (II) and theobromine (III)	Urine; Spherisorb ODS-2; 0.075 M SDS-1.5% 1-propanol; 273 nm; linearity, 2-14 $\mu\text{g/mL}$; LODs ($\mu\text{g/mL}$), 1.2 (I) and 0.4 (II, III).	29

Table 11.1 (continued)

Compounds	Samples; Stationary Phases; Mobile Phase Compositions; Detection Wavelengths; Checked Linear Ranges; Limits of Detection	Ref.
Sulfonamides: sulfacetamide, sulfadiazine, sulfamerazine, sulfathiazole, sulfamethazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfabenzamide, sulfadimethoxine, sulfadoxine and sulfisomidine	Human urine and cow milk; hydrophilic endcapped ODS at 40°C; 0.07 M SDS-6% 1-propanol.	30
Sulfonamides: sulfadiazine (I), sulfaguanidine (II), sulfamethizole (III), sulfamethoxazole (IV) and sulfathiazole (V)	Urine; Spherisorb ODS-2; 0.05 M SDS-2.4% 1-pentanol, azo dye pre-column derivatization with nitrite and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; 550 nm; LODs (µg/mL), 0.1 (I, IV), 0.2 (II, V) and 0.3 (III).	31
Alkaloid: nicotine (I) and its metabolite cotinine (II)	Urine; Econosphere CN-bonded silica; 0.2 M SDS-3% 2-propanol at pH 4.6 and 40°C; linearity, up to 3 µg/mL; LODs (µg/mL), 0.2 (I) and 0.1 (II).	32
Antiinflammatory drug: 5-lipoxygenase inhibitor Zileuton and its <i>N</i> -dehydroxylated metabolite	Urine; CN-bonded silica; 0.025 M SDS-3% 1-propanol in 0.01 M phosphate buffer at pH 3; 262 nm; linearity, 0.25-5 µg/mL; LOD, 0.1 µg/mL.	33
Steroids: hydroxycorticosterone (I), corticosterone (II), norhisterone (III), testosterone (IV), medroxyprogesterone acetate (V) and progesterone (VI)	Urine; Spheri-5 RP-18; 0.05 M SDS-9% 1-butanol; 245 nm; linearity, 0.1-10 µg/mL; LODs (ng/mL), 50 (I, II) and 100 (III, IV, V, VI).	34
Diazepam	Serum; column-switching, extraction column: ODS and 0.01 M SDS, analytical column: Adsorbosphere ODS and methanol-water 65:35 (v/v); 242 nm; linearity, 30-3000 ng/mL.	35

Table 11.1 (continued)

Compounds	Samples; Stationary Phases; Mobile Phase Compositions; Detection Wavelengths; Checked Linear Ranges; Limits of Detection	Ref.
Carbamazepine (I), chloramphenicol (II) and procainamide (III)	Serum; column-switching, extraction column: C8 (I, II), CN (III), 0.04 M SDS-14% acetonitrile (I) and 0.02 M SDS-4% acetonitrile (II, III); analytical column: Spheri-5 RP-18 and 0.04 M SDS-methanol (45:55) in 0.04 M phosphate buffer at pH 3 (I), 0.02 M SDS-methanol (72:28) in 0.02 M phosphate buffer at pH 4.6 (II) and 0.04 M SDS-acetonitrile 30:70 in 0.04 M phosphate buffer at pH 3 (III); 287 nm (I), 278 nm (II) and 280 nm (III); LODs (ng/mL), 100 (I), 50 (II) and 70 (III).	5
Anticancer drug: hexamethylene bisacetamide	Plasma and urine; column-switching, extraction column: C18 and 0.02 M SDS-10% methanol at pH 6, analytical column: C18 and methanol-water 30:70 at pH 6; 210 nm; linearity, 1-1000 µg/mL.	36
Free cortisol	Urine; column-switching, extraction column: C18 and 0.02 M SDS-18% methanol-25% 1-propanol at pH 6, analytical column: C18 and 0.02 M SDS-38% methanol-2% 1-propanol at pH 6; 254 nm; linearity, 10-1000 ng/mL; LOD, 1.2 ng/mL.	37
Anticancer drug: mitomycin-C	Plasma; column-switching, extraction column: C18 and 0.02 M SDS-10% methanol at pH 6.8, analytical column: C18 and methanol-water 30:70 at pH 6.8; 365 nm; linearity, 1-400 µg/mL.	38
Phenols: 2-aminophenol, 4-nitrophenol and phenol	Urine; column-switching, extraction column: C4 and 0.03 M CTAB-7% acetonitrile in 0.05 M 6-aminohexanoic acid at pH 5; analytical column: C18 and 0.03 M CTAB-20% acetonitrile in 0.05 M 6-aminohexanoic acid at pH 5; 300 nm.	39

The pH of the micellar mobile phase is an important factor for the analysis of ionizable drugs using nonpolar column stationary phases. Fig. 11.7 shows the chromatograms of acetylsalicylic acid ($\log K_H = 3.5$) in a serum sample at pH 3.0 and 6.5, eluted from a C18 column with a 0.08 M SDS mobile phase [20]. It can be seen that at the higher pH, the peak for this drug is not evident. The anionic solute probably eluted with the serum proteins. However, when pH was reduced to 3.5, the neutral form of the drug eluted at approximately 3.5 min, appreciably distinct from the serum components.

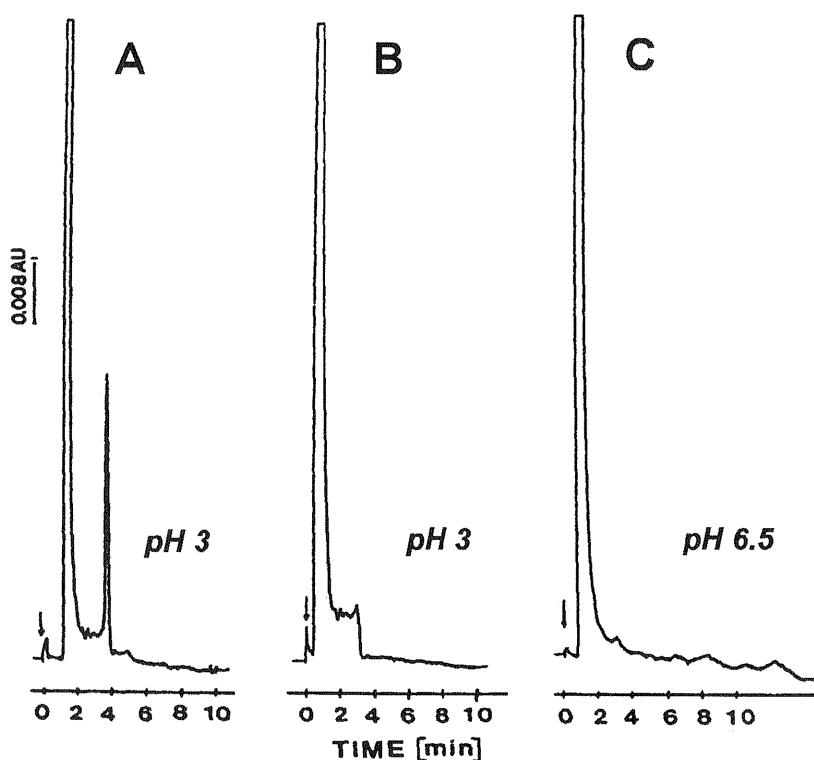
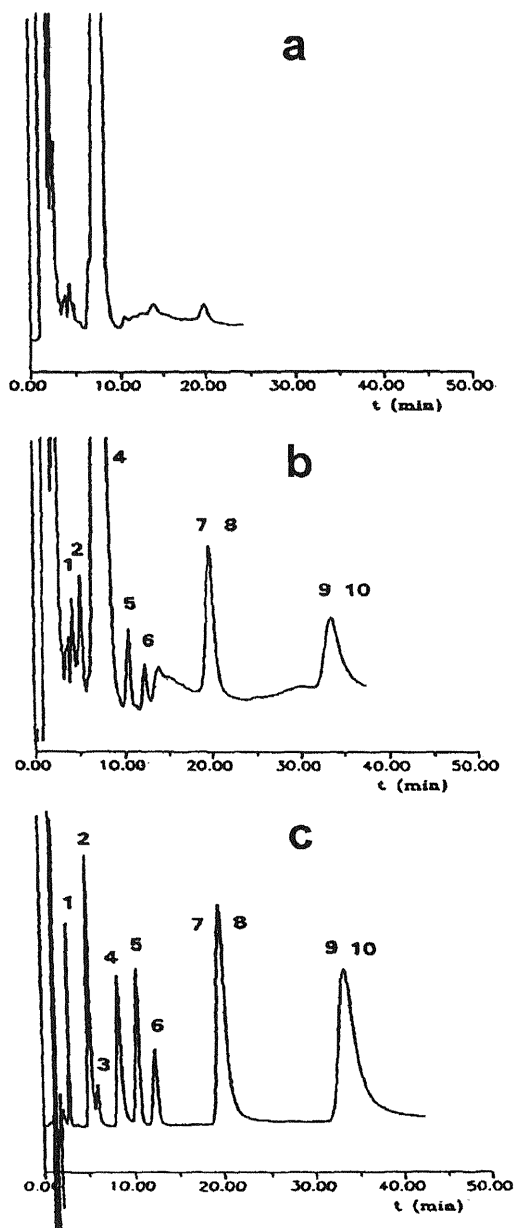


Figure 11.7 (A) and (C) Chromatograms of a serum sample containing 25 $\mu\text{g/mL}$ of acetylsalicylic acid, eluted with a 0.08 M SDS mobile phase at pH 3.0 and 6.5, respectively. (B) Chromatogram of blank serum using the same mobile phase as in (A). Reprinted from Ref. 20 with permission of the American Chemical Society.

A procedure for the determination of diuretics of different therapeutical character: high (bumetanide, ethacrynic acid, furosemide), intermediate (bendroflumethiazide, chlorthalidone, hydrochlorothiazide, xipamide) and low (acetazolamide, amiloride, spironolactone, triamterene) efficacy diuretics, and the uricosuric agent probenecid, in urine samples, illustrates a method development implying the control of pH, surfactant and modifier [23]. The greatest analytical problems in the detection of these compounds are basically their wide variety of chemical structures, functional groups and protonation constants. This implies the use of several experimental conditions for their analysis with conventional aqueous-organic mobile phases and laborious liquid-liquid or solid-liquid extraction prior to chromatographic separation. In contrast, the same micellar eluent can produce a satisfactory separation after direct injection.

Some diuretics (ethacrynic acid, bumetanide, furosemide, probenecid and xipamide) show an acid-base behavior in the pH range of 3-7. Significant changes in retention were thus observed as the pH of the anionic SDS mobile phase was modified, with longer retentions in acidic media where the compounds were protonated. In contrast, their retentions were very low at $\text{pH} > 6$ where the anionic form of the compounds dominated. The other diuretics (acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, hydrochlorothiazide, triamterene and spironolactone) did not experience changes in retention with pH. The use of SDS micellar mobile phases without a modifier, at different pH values in the range of 3-7 and in the presence of an alcohol at pH 7, did not give an adequate separation of the mixture of diuretics. Also, bumetanide, ethacrynic acid, furosemide, probenecid and xipamide were overlapped by the broad band of urine near the solvent front, at pH 6-7. Maximum separation of the peaks of these diuretics was observed at pH 4.5.

On the other hand, the retention factor of the most retained diuretic, spironolactone, at a relatively large concentration of SDS (0.15 M), was too high (retention factor, $k = 29$). An organic modifier was required for lower retention. Methanol scarcely varied the retention of the diuretics and urine matrix. In contrast, 1-pentanol excessively reduced the retention and some diuretics were overlapped by the background of urine. 1-Propanol was found to be most appropriate because of its intermediate behavior. However,

**Figure 11.8**

Chromatograms of: (a) urine matrix, (b) urine matrix spiked with a mixture of 1 $\mu\text{g/mL}$ of each diuretic and (c) an aqueous solution of the diuretics, all eluted with a 0.042 M SDS-4% 1-propanol mobile phase in phosphate buffer at pH 4.5. Compounds: (1) furosemide, (2) chlor-thalidone, (3) ethacrynic acid, (4) bendroflumethiazide, (5) probenecid, (6) bumetanide, (7) amiloride, (8) xipamide, (9) spironolactone and (10) triamterene. Reprinted from Ref. 23 with permission of Elsevier.

acetazolamide and hydrochlorothiazide appeared at the solvent front, overlapped by the broad band of urine.

Further optimization of mobile phase composition (SDS and 1-propanol concentration) was made using the method developed by Torres Lapasió et al. [40], assisted by the *MICHRON* software (see attached CD ROM). Maximum resolution corresponded to 0.042 M SDS-4% 1-propanol. Fig. 11.8 shows the experimental chromatogram for this mobile phase of a urine sample spiked with a mixture of the diuretics, together with a similar chromatogram obtained with an aqueous solution. The retention times for both chromatograms were similar. The peaks of amiloride and xipamide, on the one hand and triamterene and spironolactone on the other, appeared mutually overlapped in the chromatograms. The peak of bendroflumethiazide was overlapped by the prominent peak of urine.

Opportunities to further reduce matrix interferences through chemometric techniques and diode array detection present challenges for future studies.

V.3. Comments on Selected Applications

A good example of the capability of MLC, in the separation of mixtures of drugs, is given by the screening of twelve sulfonamides in human urine and cow milk with an SDS-2-propanol mobile phase and a hydrophilic endcapped C18 column (Fig. 11.9) [30]. Using a 0.07 M SDS-6% 1-propanol eluent at pH 3.0, isocratic separation of the sulfonamides was achieved within 15 min.

As commented above, the use of a surfactant different from SDS is anecdotal in direct injection MLC procedures. For this reason, the comparison of the determination of a drug such as theophylline (1,3-dimethylxanthine), using two different surfactants, is interesting. Habel et al. [9] reported a procedure for the direct determination of this drug in human serum by using a μ -Bondapak phenyl column, a micellar mobile phase of the zwitterionic surfactant C₁₂ DAPS (10^{-3} M) containing 3% 1-propanol and UV detection at 273 nm. Later, Pérez Martínez et al. [29] developed a procedure for theophylline, caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine), in urine, with a Spherisorb C18

column, a 0.075 M-1.5% 1-propanol eluent and UV detection at the same wavelength. The two surfactants gave similar results, since theophylline eluted in both cases in 5 min, sufficiently apart from the broad band of the matrix, the chromatographic peak was almost symmetric and the LOD was near 0.5 $\mu\text{g/mL}$. With the SDS mobile phase, the peaks of caffeine and its metabolites were well separated. Certainly, the good characteristics and availability of SDS will make it difficult to be replaced in the future by other surfactants in liquid chromatographic drug analysis.

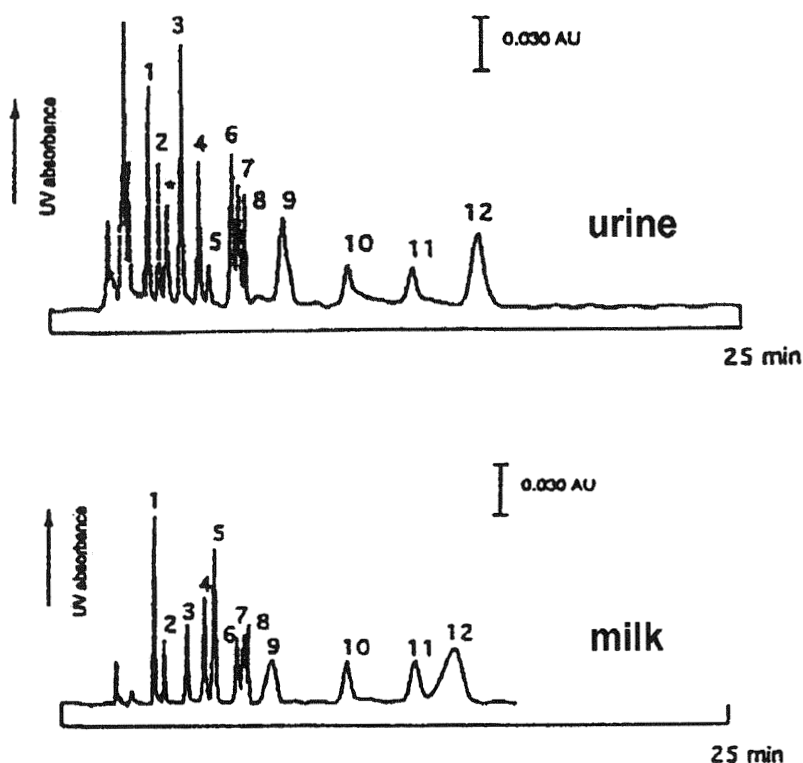


Figure 11.9 Separation of sulfonamides in: human urine (top) and cow milk (bottom). Mobile phase was 0.07 M SDS-6% 1-propanol in 0.02 M phosphate buffer at pH 3.0. Compounds: (1) sulfacetamide, (2) sulfadiazine, (3) sulfamerazine, (4) sulfathiazole, (5) sulfamethazine, (6) sulfamethoxypyridazine, (7) sulfachloropyridazine, (8) sulfamonomethoxine, (9) sulfabenzamide, (10) sulfadimethoxine, (11) sulfaquinoxaline and (12) sulfisomidine. Reprinted from Ref. 30 with permission of Elsevier.

Micellar mobile phases containing modifiers permit the adequate separation of mixtures of solutes of different hydrophobicity, with isocratic elution. However, for samples containing compounds with a wide range of retention, gradient elution can be used to hasten the elution of strongly retained compounds. This will reduce the analysis time and improve greatly the sensitivity of detection. In fact, rapid gradient capability is another advantage of MLC, where the stationary phase must not be reequilibrated with the original mobile phase composition after each run. However, the reported MLC procedures using gradient elution are few. One example is the determination of the diuretic bumetanide in serum, using a mobile phase of SDS-3% 1-propanol with phosphate buffer at pH 3.5 [10], where the initial SDS concentration of 0.1 M was held until the peak of bumetanide was completely eluted (7 min) and then, the concentration of SDS was increased to 0.2 M over 5 min to ensure that any adsorbed serum components were washed from the column. Another application is the determination of thiazide diuretics and furosemide, where an SDS gradient was used to accelerate the elution of furosemide [24].

Mixed surfactant mobile phases can be useful to improve the selectivity required in difficult separations [18]. Although hydrochloro thiazide was well separated from the urine matrix with a Brij-35 mobile phase, no resolution could be achieved between this diuretic and its hydrolysis product 5-chloro-2,4-disulfamoylaniline, which is formed in aqueous solution upon standing at room temperature. In contrast, baseline separation was obtained between the two compounds with SDS mobile phases. However, SDS eluents did not provide separation of the diuretic from the prominent peak in urine. The optimized mobile phase contained 0.004 M SDS (below its cmc), in addition to 0.02 M Brij-35. The small amount of SDS was added to obtain resolution between the diuretic and its hydrolysis product, without compromising the separation of the drug from the urine background.

V.4. Precolumn Derivatization

UV detection is the most available mode in liquid chromatography, its sensitivity is however insufficient for some trace amount determinations. Therefore, other detection modes have been utilized to enhance this

capability. The use of precolumn derivatization to improve the sensitivity and selectivity in MLC determinations is commented next. Other techniques are described in Chapter 12.

Most amino acids cannot be detected by direct UV monitoring. A procedure was developed for the determination of a mixture of these compounds (proline, glutamine, threonine and tyrosine) in urine, by precolumn formation of the Cu(II) complexes and detection at 235 nm, using a 0.03 M SDS-8% 1-propanol mobile phase at pH 5.5 [25].

Otherwise, the high background at the beginning of the chromatogram of a physiological sample, that appears when the detection is performed at 230-280 nm, is eliminated in the visible region. To exploit this fact, the azo dyes of several sulfonamides (sulfamethizole, sulfaguanidine, sulfamethoxazole, sulfadiazine and sulfathiazole) with the Bratton-Marshall reagent [*N*-(1-naphthyl)ethylenediamine dihydrochloride, NED] were formed in micellar solution before injection in an MLC system. The derivatization reaction is rapid, can be automated and yields high molar absorptivities at large wavelengths. The elution was performed with 0.05 M SDS-2.4% 1-pentanol and the detection at 488 nm [31]. The chromatogram of urine matrix treated with the derivatization reagents gave only one peak at 13.5 min, which corresponded to an unknown endogeneous compound that probably formed an azo dye. This peak did not interfere with the determination of the sulfonamide azo dyes, which eluted at shorter retention times.

The described method gathered the advantages of precolumn derivatization and chromatography with micellar mobile phases. The derivatization of the sulfonamides increased the retention by reducing the polarity of the drugs and improved the signal-to-noise ratio and the resolution of the chromatograms. This produced the adequate separation and detection of the sulfonamides in urine.

Another example of an MLC procedure with precolumn derivatization is the determination of thiazide diuretics, after hydrolysis and formation of the NED azo dyes [24]. The thiazide diuretics originated only two different arylamines by hydrolysis, depending on the existence of a -Cl or -CF₃ substituent in the thiazide nucleus. Therefore, only two peaks were observed in the chromatograms for mixtures of these compounds (Fig.

11.10). Underivatized diuretics could not be detected, especially those with a -Cl substituent, due to overlapping with the band of the proteins and the prominent peak of an endogeneous compound, in the physiological sample.

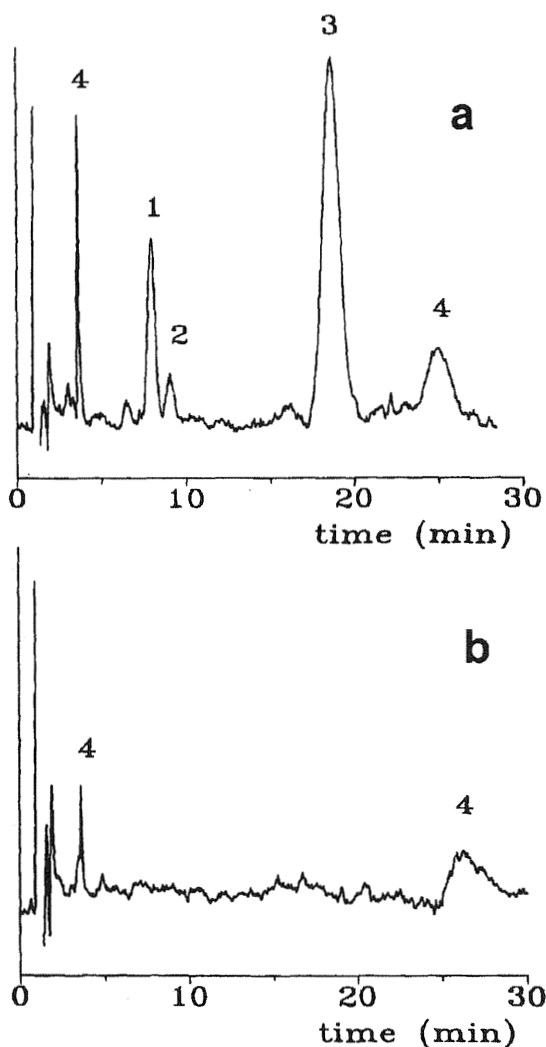


Figure 11.10 Chromatograms of: (a) spiked urine containing: (1) hydrochlorothiazide, (2) hydroflumethiazide, (3) furosemide and (4) endogeneous compounds; and (b) urine blank. The samples were subjected to hydrolysis and derivatization with the Bratton-Marshall reagent. Reprinted from Ref. 24 with permission of Elsevier.

VI. SCREENING OF ILLEGAL DRUGS IN SPORT

VI.1. Banned Drugs

MLC has been used to analyze physiological samples for assay of drugs illegally used in sport. The use of performance enhancing drugs by sportsmen and sportswomen is recognized today as one of the key problems in sport, toxicology and sport medicine [41]. Competitors are frequently pressured to beat marks and succeed which, unfortunately, leads to drug misuse to enhance their performance. Doping practice in modern sport has increased through the years. The extent of drug use is unknown, although some indicators show that it is wider than just a few isolated cases (*e.g.* Tour de France 1998). In addition to ethical considerations, several of the classes of drugs routinely abused can cause adverse effects in the organism, including changes in blood viscosity, cardiac function and peripheral resistance. These changes may be deadly in combination with exercise-induced physiological alterations, such as those related with heat load, electrolyte balance, fluid balance and metabolic rate.

The use of performance-enhancing drugs constitutes a banned practice in most official athletic competitions. The experience acquired in the controls performed by several international organizations has led to a list of banned drugs, which is updated continuously. Five pharmacological categories of doping drugs are considered by the Medical Commission of the International Olympic Committee (IOC): stimulants, narcotic analgesics, anabolic steroids, β -blockers and diuretics [41]. The availability of analytical methods to determine unequivocally these compounds and their metabolites in physiological fluids has been one of the main reasons for inclusion of these banned classes. Thus, although it was well known that anabolic steroids were being misused in sport before 1960, the IOC did not ban the class until 1975, when suitable methods of testing had been developed.

VI.2. Analytical Methodologies

Urine is the physiological fluid of choice for doping control because of the convenience in taking the samples, relative simplicity of its composition,

compared with other body fluids, and accumulated experience. The complexity of the dope problem has forced the development of a variety of advanced analytical methods, in order to detect and characterize any kind of banned drugs. These include gas chromatography, liquid chromatography, hyphenated gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry, fluorescence polarization and immunoassay methods. Most methods require some degree of sample conditioning prior to detection, starting with the extraction of the drug from the urine matrix. For compounds excreted as conjugates in urine, hydrolysis of the metabolites is required. Gas chromatography with conventional detectors or in combination with mass spectrometry requires additional sample derivatization, to increase the volatility of the drugs. Even compounds excreted free in urine need liquid-liquid or solid-liquid extraction before this chromatographic analysis.

Several aspects of the official methodology should be considered in doping control. Screening procedures need to be based only on semi-quantitative approaches, since the purpose is detection. Suspect samples should be submitted for further confirmatory analysis by a technique based on a different analytical principle, before making such an important decision as to whether somebody has used a drug before competition. Increasing sample throughput is becoming extremely important in the sport-oriented laboratories. More than 200 drugs and metabolites must be tested for a variety of doping agents in sporting events. Laboratories are receiving more and more samples and are expected to keep turn-around times to a minimum. In this context, methods needing little or no sample preparation, resulting in a reduction of analysis time, are of great interest.

Among the different testing methods approved for illegal drugs in the Olympics, only a few liquid chromatographic procedures are included. The tedious sample preparation and lengthy analysis times preclude the general use of the existing conventional HPLC procedures for rapid screening. Two Spanish groups headed by Laserna and García Alvarez-Coque studied the possibility of MLC in the screening of illegal drugs [26,42,43]. Two important advantages of this technique are the direct injection of urine, and the possibility of determining, from a single injection, compounds of different chemical structures. In MLC, the rapid elution of proteinaceous material of urine opens a large window for the detection of banned drugs. However,

many drugs are excreted as conjugates in urine and the advantages of direct sample injection methods are of decreased relevance. Also, unfortunately, the availability of real urine samples from doped athletes is limited and not always can a real sample analyses be made in the development procedures. Further evaluation of the methods is needed in order to implement it as a complementary methodology for doping control.

VII. PREVIOUS SEPARATION OF THE DRUGS

VII.1. Extraction Procedures

Quantitation at low concentration levels of some drugs by MLC with direct injection may not be feasible for several reasons: (i) the drug peak is overlapped by the protein band and peaks of endogeneous compounds in the physiological matrix, (ii) the drug peak is overlapped by the peaks of other drugs consumed by the individual or (iii) the LOD is insufficient. The MLC approach improves column lifetime and provides a mechanism for release of strongly protein-bound drugs. However, it implies moderate efficiency due to poor mass transfer and, with large injection volumes, the inefficiency is amplified and the high amounts of proteins in the sample can deteriorate the chromatographic column. As with any singular dimension approach with a small injection volume and the lack of an enrichment step, the sensitivity of the determination is inadequate for many applications.

Some authors have considered the MLC approach still interesting for the determination of drugs in physiological fluids, when a previous separation step is required. It is expected that the combined selectivity of extraction procedures and MLC will provide wide resolution power to avoid presumable restrictions. Thus, the antipyrine metabolites 4-aminoantipyrine, 4-methylaminoantipyrine and 4-formylaminoantipyrine were well separated in plasma samples and eluted in less than 5 min with a 0.1 M-2.5% 1-pentanol mobile phase and C18 column. Extraction with methylene chloride [27], or through disposable C18 bonded porous silica cartridges [28], with methanol as an eluent, were necessary to remove the metabolites from the

matrix. In the procedures, the organic extraction solvents were evaporated to dryness and reconstituted with mobile phase before injection into the chromatographic system. Furosemide was used as an internal standard.

Also, previous to the MLC separation, chlorthalidone was extracted into diethyl ether-2-propanol from plasma, using xipamide as an internal standard [12], catecholamines were extracted from urine through alumina columns and eluted with 0.5 M perchloric acid [44] and clenbuterol in urine was eluted from a cation-exchange sorbent with the same solvent used as mobile phase (0.1 M SDS-12% 1-butanol) [45].

The determination of steroids is difficult due to their high hydrophobicity and very low concentration in physiological samples (in the low ng/mL level). A mobile phase of SDS and 1-pentanol permitted the separation and adequate elution of a mixture of medroxyprogesterone, methyltestosterone, medroxyprogesterone acetate, dydrogesterone, progesterone, testosterone propionate and testosterone enanthate [46], however, the LOD of UV monitoring was not enough for its quantitation in urine samples, when direct injection was made. Attempts were also unsuccessful to reach an adequate LOD by using sensitized terbium fluorescence detection [47]. Thus, for these analyses, a previous separation is imperative.

VII.2. Use of Column-Switching with Micellar Clean-up

The lack of an enrichment step precludes simple one-dimensional chromatographic separations with direct injection from providing the required LODs for many determinations. Adopting a multidimensional approach can improve the sensitivity by allowing large injection volumes (*e.g.*, 100 μ L) and yet permits trace enrichment and peak compression through solvent focusing. This approach demonstrates a practical use of MLC expanded beyond the singular dimensional chromatographic process.

Some reported multidimensional procedures make use of the advantages of conventional RPLC (high column efficiency) and MLC (extended column life with direct injection of physiological fluids, extraction of protein-bound drugs and the ability to finely control the elution pattern on the clean-up column) [5,35,48]. The procedures operate with a consumable

extraction column that can last for several hundred injections. The first chromatographic dimension provides the sample extraction and clean-up with a micellar SDS mobile phase. The second dimension, coupled on-line to the first, utilizes conventional reversed-phase media and organic solvents for the analytical separation. With the proteinaceous material removed in the extraction step, any reversed-phase packing material is compatible with the system. The three column-switching configurations shown in Fig. 11.1 have been used.

Multicomponent analysis is facile under a set of well defined conditions: (i) the compounds separate from excluded material, (ii) elute off the extraction column in a single band and (iii) are separable on the analytical column, (iv) the mechanism of separation differs (ideally) in each of the columns and (v) separation and detection are adequate. The micellar eluent is selected to provide the proper retention characteristics, but generally requires only modest modification for differing applications. Irregardless of the type of bonded phase, the excluded components from the extraction column should occupy the first minutes of the chromatogram with the micellar eluents used. Also, the analytical phase should be more retentive for the drug component than the extraction phase with the eluent used during the purge phase (see Fig. 11.1). Because of trace enrichment, the purity of all reagents, primarily SDS, is especially critical.

Posluszny et al. [5], in one of the first procedures, cut the drug onto the analytical column with micellar mobile phase as it eluted off the tail of the exclusion front. This style of recovery had three significant disadvantages that markedly impacted the selectivity: (i) endogeneous substances were eluted onto the analytical column, (ii) the micellar mobile phase was drastically different from the analytical mobile phase, resulting in a large baseline shift since as much as 4 mL might be cut onto the analytical column and (iii) the relatively weak eluting power of the micellar mobile phase resulted in the retention and build-up of hydrophobic substances on the analytical column.

These disadvantages were overcome by providing for a more selective elution of the extraction column [48]. This was achieved in the following stepwise manner: (i) the physiological sample was injected under micellar conditions (the micellar solvent and column were selected to ensure

significant retention of the drug), (ii) after clearance of the proteinaceous material, the drug was eluted with a nonmicellar recovery mobile phase (the extraction and analytical columns were placed in series and the drug recovered onto the analytical column), (iii) the valve was switched to the analytical mobile phase and the separation was performed on the analytical column and (iv) simultaneously with step (iii), the extraction column was washed and reequilibrated with micellar mobile phase.

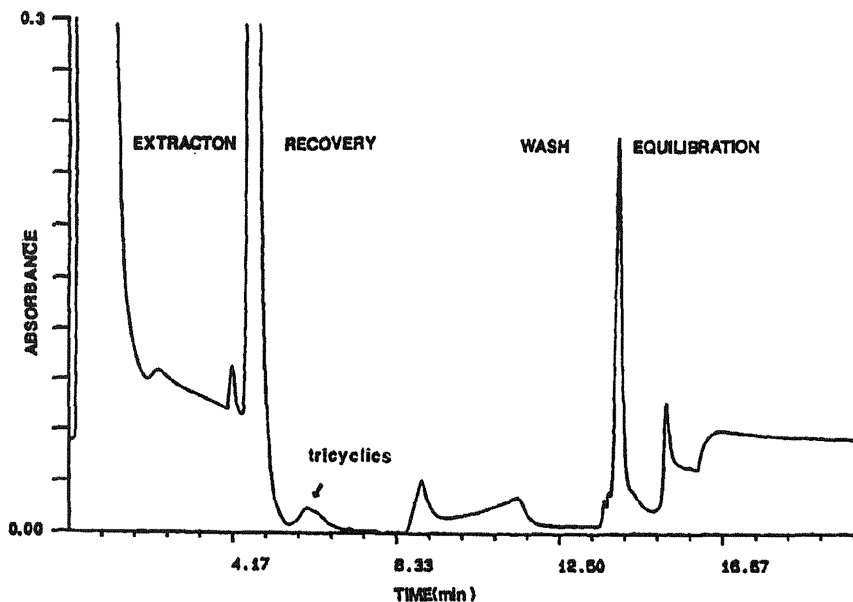


Figure 11.11 Chromatogram of the extraction column for tricyclic antidepressant separation in plasma. Extraction column: 4 cm x 4.6 mm i.d. polystyrene-divinylbenzene, 10 μ m spherical polymer. Extraction eluent: 0.06 M SDS-8% (v/v) acetonitrile-0.2% triethylamine in 0.02 M phosphate buffer at pH 7.2. Recovery eluent: 0.08 M SDS-56% acetonitrile-12% methanol in 0.09 M phosphate buffer at pH 3.0. Wash eluent: 0.06 M SDS-90% acetonitrile. Reprinted from Ref. 48 with permission of Elsevier.

This protocol resulted in the following ordering of mobile phases flowing through the extraction column: (i) extraction solution (the micellar solvent that washes endogeneous material off the column while permitting analyte retention), (ii) recovery solution (the solvent that elutes the analyte off the extraction column onto the analytical column) and (iii) wash solution (a strong solvent employed to clean-up the extraction column). The recovery

solvent is usually an aqueous dilution of the analytical mobile phase. This measure generally provides for solvent focusing on the head of the analytical column. The end result is a dramatic sharpening of the peaks of interest both on the extraction column, as well as on the analytical column. Both sensitivity and selectivity are improved.

A column-switching separation of an injection of 500 μL of blood plasma containing 270 ng/mL of a mixture of the tricyclic antidepressant drugs, doxepin, desipramine, nortriptyline, imipramine and amitriptyline, is shown in Figs. 11.11 and 11.12 [48]. Figure 11.11 illustrates the selective recovery of the tricyclics from a polymeric extraction column. The five components are separated as a single peak from the solvent front of the recovery mobile phase. The recovery is accomplished by a pH change: the extraction solvent pH was adjusted to 10 with triethylamine, while the recovery solvent was formulated without the base to ensure that nothing of

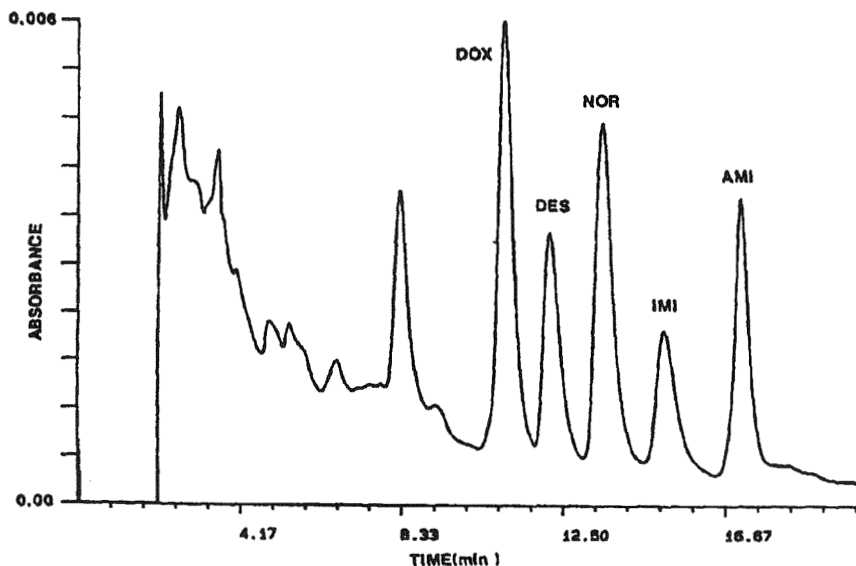


Figure 11.12 Multidimensional chromatogram of tricyclic antidepressants in blood plasma. Extraction column and extraction, recovery and wash eluents as in Fig. 11.11. Analytical column: Spheri-5, RP-18, 22 cm x 4.6 mm i.d. Analytical eluent: 0.08 M SDS-56% (v/v) acetonitrile-32% methanol-0.08% triethylamine in 0.09 M phosphate buffer at pH 3.0. Compounds: doxepin (DOX), desipramine (DES), nortriptyline (NOR), imipramine (IMI) and amitriptyline (AMI). Reprinted from Ref. 48 with permission of Elsevier.

the high-pH extraction solvent passed onto the silica-based analytical column. Triethylamine was again added to the analytical mobile phase to minimize peak tailing.

Other applications of column-switching are given in Table 11.1. In some cases, SDS was added to the analytical mobile phase to minimize artifacts from the switching process or to act as an ion-pair reagent for the analyte. At the high levels of organic modifiers present in the solutions, micelles if still present are severely altered compared to more aqueous media. Also, a specified amount of organic modifier was usually added to the micellar extraction mobile phase to increase the eluting power of the otherwise relatively weak micellar solution. Saturation of the stationary phase in the extraction column was maintained by the addition of surfactant to the wash solution.

Both the extraction and analytical column chromatography should be developed independently. Afterwards, the multidimensional system will be integrated with a column-switching valve. Sample throughput can be increased by reducing the time necessary for washing and re-equilibrating the extraction column and making use of faster analytical chromatography. Also, one sample can be loaded on the precolumn while another is being chromatographed on the analytical column.

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Enhanced Detection in Micellar Medium

I. INTRODUCTION

The impressive ability of micelles to manipulate the microenvironment experienced by solubilized molecules may greatly alter their properties. In previous chapters, aqueous solutions of micelles have been shown to produce unique, effective separations when used as mobile phases in liquid chromatography. But another aspect that is not adequately considered is the effects on detection.

In recent years, there has been a rapid growth in the number of publications that report the use of surfactant monomers or micelles to improve the analytical performance of various spectroscopic (UV-visible spectrophotometry, fluorimetry, phosphorimetry, chemiluminescence and atomic spectroscopy), and electrochemical (especially amperometry) methods [1]. The unique properties of surfactants have been recognized as being very helpful to overcome many problems associated with the use of organic solvents in these methods. Surfactant-modified procedures yield sensitivity and/or selectivity improvements in determinations commonly performed in homogeneous solution, whereas certain analytical methods (such as room-temperature phosphorescence in solution) can be exclusively conducted in organized media.

Changes in UV-visible absorption maxima (intensity and wavelength) can occur by the addition of surfactants. Several analytical procedures developed in Micellar Liquid Chromatography (MLC) with UV-visible detection were presented in Chapters 10 and 11. Most procedures were applied to the analysis of pharmaceutical preparations and physiological fluids. Although it was obvious that several compounds experienced

Table 12.1 Experimental Characteristics of Some MLC Procedures for the Determination of Compounds using Micellar Enhanced Detection

Compounds	Samples; Detection Mode; Stationary Phases; Mobile Phase Compositions; Checked Linear Ranges; Limits of Detection	Ref.
PAHs: Anthracene (I), 1,2-benzanthracene (II), benzo[<i>e</i>]pyrene (III), biphenyl (IV), fluoranthene (V), naphthalene (VI) and pyrene (VII)	Alkyl nitrile; fluorescence; 0.024 M SDS; LODs ($\mu\text{g/mL}$), 0.2 (I, III, IV), 0.5 (II), 2.5 (V), 0.3 (VI) and 0.25 (VII).	2
PAHs: acenaphthene (I), acenaphthylene (II), anthracene (III), benzo[<i>a</i>]fluorene (IV), biphenyl (V), fluoranthene (VI), fluorene (VII), 2-methylanthracene (VIII), naphthalene (IX), phenanthrene (X), pyrene (XI) and triphenylene (XII)	C18; fluorescence [λ_{exc} (nm) = 360 (II, IV), 338 (XI) and λ_{em} (nm) = 425 (II, IV), 400 (XI)]; 0.035 M SDS-3% 2-propanol; LODs (ng/mL), 100 (II), 270 (IV) and 1.7 (XI).	3
PAHs: acenaphthylene (I), anthracene (II), benz[<i>a</i>]anthracene (III), benzo[<i>a</i>]fluoranthene (IV), benzo[<i>g,h,i</i>]perylene (V), benzo[<i>a</i>]pyrene (VI), benzo[<i>e</i>]pyrene (VII), chrysene (VIII), dibenz[<i>a,c</i>]anthracene (IX), dibenz[<i>a,h</i>]anthracene (X), fluoranthene (XI), fluorene (XII), 9-methyl-anthracene (XIII), naphthalene (XIV), perylene (XV), phenanthrene (XVI) and pyrene (XVII)	Sea water, sediments and biota; fluorescence with variable programmed wavelength; Nova-Pak C18; 0.15 M SDS-15% 2-propanol; linearity ($\mu\text{g/mL}$), 0.05-3.0 (III), 0.02-2.2 (IV), 0.03-4.0 (V), 0.06-1.5 (VI), 0.1-3.0 (VIII), 0.04-1.2 (IX), 0.2-7.5 (XI), 0.05-1.2 (XII), 0.1-12.0 (XIII), 0.1-4.0 (XIV), 0.02-2.0 (XV), 0.05-3.2 (XVI) and 0.04-1.8 (XVII); LODs (pg), 39 (III), 17 (IV), 29 (V), 11 (VI), 20 (VIII), 9 (IX), 58 (XI), 5 (XII), 97 (XIII), 21 (XIV), 13 (XV), 20 (XVI) and 10 (XVII).	4
Quinidine	Serum; fluorescence (λ_{exc} = 336 nm, λ_{em} = 370 nm); Supelcosil LC-CN; 0.10 M SDS; LOD, 0.3 $\mu\text{g/mL}$.	5

Table 12.1 (continued)

Compounds	Samples; Detection Mode; Stationary Phases; Mobile Phase Compositions; Checked Linear Ranges; Limits of Detection	Ref.
Codeine (I), morphine (II), propranolol (III), quinidine (IV) and quinine (V)	Serum and urine; fluorescence ($\lambda_{exc} = 215$ nm and $\lambda_{em} = 300$ nm); μ Bondapak C18 and Supelcosil LC-CN; 0.02-0.05 M SDS-10% 1-propanol; linearity (μ g/mL), 0.5-2.0 (I), 0.4-1.2 (II), 0.04-1.2 (III), 0.2-1.0 (IV) and 0.2-1.0 (V); LODs (μ g/mL), 0.3 (I, II), 0.01 (III) and 0.03 (IV, V).	6
Alprenolol, bendroflumethiazide, furosemide, nadolol, phenylephrine, propranolol and triamterene	Spheri-5 RP-18; fluorescence after isothiocyanate derivatization; 0.1 M SDS-7% 1-pentanol.	7
β -Blockers: acebutolol (I), atenolol (II), celiprolol (III), labetalol (IV), metoprolol (V), nadolol (VI) and propranolol (VII)	Urine; fluorescence [$\lambda_{exc} = 230$ nm and $\lambda_{em} = 440$ nm (I, III, IV), 300 nm (II, V, VI) and 340 nm (VII)]; Spherisorb ODS-2; 0.1 M SDS-15% 1-propanol-1% triethylamine-0.02 M phosphate buffer at pH 3; linearity (μ g/mL), 0.1-4.2 (I), 0.05-2.1 (II), 0.5-4.2 (III), 0.1-0.9 (IV), 0.05-0.5 (V), 0.05-2.2 (VI) and 0.004-0.1 (VII); LODs (ng/mL), 30 (I), 19 (II), 200 (III), 20 (IV), 16 (V), 8 (VI) and 3 (VII).	8
Acyclovir	Serum and plasma; fluorescence ($\lambda_{exc} = 285$ nm and $\lambda_{em} = 370$ nm); Separon SGX C18; 0.05 M SDS-0.05 M phosphate at pH 2.05; LOD, 0.08 μ g/mL.	9
Plant growth regulators: indol 3-yl acetic acid (I), 2-(1-naphthyl) acetic acid (II), indol 3-yl propionic acid (III), 2-(2-naphthyl) acetic acid (IV), indol 3-yl butyric acid (V), 2-(1-naphthyl) acetamide (VI) and indol 3-yl acetic acid ethyl ester (VII)	Plant extracts; fluorescence ($\lambda_{exc} = 281$ nm and $\lambda_{em} = 340$ nm); Lichrospher alkylnitrile; 0.010 M SDS with pH gradient elution; linearity (μ g/mL), 0.01-4 (I), 0.01-8 (II, III), 0.02-8 (IV), 0.01-20 (V), 0.02-20 (VI) and 0.05-20 (VII); LODs (μ g/g), 0.3 (I, III, V), 0.8 (II), 1.0 (IV) and 1.1 (VI, VII).	10
Aluminium	Serum; fluorescence ($\lambda_{exc} = 370$ nm and $\lambda_{em} = 504$ nm); Capcell Pak MF pH-1; 0.010 M SDS-20% acetonitrile (precolumn kinetic differentiation mode formation of the aluminium 8-quinolinol chelate); LOD, 1 ng/mL.	11

Table 12.1 (continued)

Compounds	Samples; Detection Mode; Stationary Phases; Mobile Phase Compositions; Checked Linear Ranges; Limits of Detection	Ref.
Acetazolamide, amphetamine, atenolol, chlorthalidone, furosemide, heptaminol, pemoline and phenylephrine	Spheri-5 RP-18; laser-induced fluorescence (argon laser, $\lambda_{\text{exc}} = 488$ nm) after isothiocyanate derivatization; 0.1 M SDS.	7
Steroids: bolasterone (I), cortisone (II), methyltestosterone (III), progesterone (IV), testosterone (V) and testosterone acetate (VI)	Urine; sensitized terbium fluorescence ($\lambda_{\text{exc}} = 245$ nm and $\lambda_{\text{em}} = 547$ nm); C18; 0.10 M SDS-20% acetonitrile-0.01 Tb(NO ₃) ₃ ; linearity, 2 orders of magnitude; LODs (ng/mL), 10 (I, IV, VI) and 50 (III, V).	12
PAHs: Biphenyl, naphthalene and pyrene	Alkyl nitrile; phosphorescence; oxygen free 0.016 M SDS-0.008 M Tl(III) dodecyl sulfate.	2
PAHs: Biphenyl (I), naphthalene (II), β -naphthol (III) and phenanthrene (IV)	Propylcyano; phosphorescence [$\lambda_{\text{exc}} = 280$ nm (III), 260 nm (I, IV) and $\lambda_{\text{em}} = 550$ nm]; 0.15 M SDS-Tl(III) dodecyl sulfate (70:30); linearity, 2-3 orders of magnitude; LODs (ng), 20 (I), 8 (III) and 3 (IV).	13
Alkyltin compounds: Monomethyltin trichloride (I), dimethyltin dichloride (II), trimethyltin chloride (III), triethyltin bromide (IV) and tripropyltin chloride (V)	Spherisorb ODS-2; ICP-MS; 0.02 M SDS (I-III), 0.1 M SDS (III-V); linearity, 3.5 orders of magnitude; LODs (pg tin), 46 (I), 26 (II), 27 (III, 0.02 M SDS), 126 (III, 0.1 M SDS), 51 (IV) and 111 (V).	14

Table 12.1 (continued)

Compounds	Samples; Detection Mode; Stationary Phases; Mobile Phase Compositions; Checked Linear Ranges; Limits of Detection	Ref.
Alkyltin compounds: dibutyltin (I), tributyltin (II), diphenyltin (III) and triphenyltin (IV)	Seawater; ICP-MS; YMC-Pack FL-C4; 0.04 M tris(hydroxymethyl)amino methane dodecyl sulfate-20% ethanol-3% acetic acid (v/v); LODs (pg), 35 (I), 27 (II), 52 (III) and 25 (IV).	15
Arsenic compounds: dimethylarsenic acid (I), monomethylarsonic acid (II), As(III) (III) and As(V) (IV)	Urine; ICP-MS; Hamilton PRP-1; 0.05 M cetyltrimethylammonium bromide-10% 1-propanol-0.02 M borate at pH 10.2; linearity, 3 orders of magnitude; LODs (pg), 90 (I) and 300 (II, III, IV).	16
Phenols: <i>p</i> -bromophenol, catechol, <i>p</i> -chlorophenol, hydroquinone, <i>o</i> -nitrophenol, <i>p</i> -nitrophenol, phenol and resorcinol	C8; amperometry with glassy carbon electrode; gradient elution: solvent A (0.05 M SDS-3% 1-propanol-phosphate buffer at pH 2.5-NaClO ₄ added to balance the conductivity) to solvent B (0.112 M SDS-3% 1-propanol-phosphate buffer at pH 2.5) in 15 min.	17
Dopamine	Urine; amperometry with glassy carbon electrode; Micropak ODS; 0.01 M SDS-3% 1-propanol-0.02 M citrate at pH 4.15-0.001 M EDTA; linearity, 1 ng/mL-10 µg/mL; LOD, 4 pg.	18
Peptide 520	Human plasma; photolytic amperometry with glassy carbon electrode; Supelco LC-18; 0.232 g sodium octyl sulfate-22% methanol-78% phosphate buffer at pH 6.5.	19
Acetaminophen	Urine; amperometry with a wall-jet cell and carbon fibre microelectrode; Nucleosil C18; 0.05 M SDS-3% 1-propanol; linearity, 0.02-70 µg/mL; LOD, 0.02 µg/mL.	20

enhanced absorption in the micellar medium, this question was not addressed in most analytical reports.

In this chapter, the features of other detection methods that have been utilized in MLC: conventional and sensitized fluorimetry, room-temperature phosphorimetry, inductively coupled plasma hyphenated with mass spectrometry, and amperometry, are examined. Table 12.1 gives details of some reported procedures. Most of them appeared during the 90s, when the development of applications in MLC increased. The study and use of new detection systems can result in enhanced flexibility and efficiency for the separation analyst.

II. ENHANCED FLUORESCENCE DETECTION

II.1. Effect of Micelles on Sensitivity

The heterogeneous chemical microenvironment provided by micelles influences excited-state equilibria by imposing additional constraints and pathways on the molecule's behavior. Fluorescence parameters such as excitation and emission wavelengths, quantum yields, fluorescence lifetimes and relaxation processes for the excited states may change. The differences observed result frequently in increased fluorescence intensities and/or reduced interference from impurities. From an analytical point of view, these phenomena can be very useful. Micellar-enhanced fluorescence is a method that seems very promising and interest in it is steadily increasing with a view to developing more sensitive and convenient methods for the determination of molecules and metal ions. Some MLC procedures have taken advantage of this method.

The observed enhancement in emission intensity in micellar systems must derive from an increase in either solute molar absorptivity at the exciting wavelength and/or quantum yield, compared with that in bulk solvent alone. The rate constants for deactivation of the excited states by radiationless processes are also significantly reduced when the

solutes are in the presence of micelles. The reason is that the organic compounds are effectively compartmentalized when they partition to or bind the micellar system and the emitting excited state is thus protected from quenching. The bound solutes are probably much more restricted (less mobile) in such an environment, compared with the situation in bulk solvent. In addition, the micropolarity is reduced and the microviscosity increased [1, 21].

The pH of the solution is also an important parameter that will influence the luminescence characteristics of organic species that exhibit acid-base properties. In many instances, the chemical and physical properties of electronically excited molecules differ markedly from those of the ground-state molecules, because of the different electronic distribution. Therefore, most of the excited molecules show protonation constants ($\log K_H$) which differ greatly from those measured in their ground states. Differences in $\log K_H$ of more than 6 units have been observed in a variety of compounds. As for the ground state, acid-base equilibria in the excited state are drastically altered by the surfactant aggregates, which can result in a further increase in sensitivity.

The gain in sensitivity is usually measured by the micellar enhancement factor, defined as the ratio between the fluorescence intensity in micellar solution and in homogeneous solvent, at the same fluorophor concentration. The observed intensities are usually many times greater than in the corresponding homogeneous media. Thus, the fluorescence of acyclovir (a drug with a considerable activity against viruses of the herpes group) was increased by a factor of five and the emission maxima was shifted by about 15 nm to the short-wavelength region, by addition of sodium dodecyl sulfate (SDS) to the aqueous solution [9]. In fact, a slight increase in the relative intensity of fluorescence with SDS concentration was already visible in the concentration region below the critical micellar concentration (cmc) (Fig. 12.1). This was caused by the influence of SDS on the protonation constant of acyclovir. The constant increased and thus the formation of the protonated compound which is responsible of the fluorescence signal.

In contrast, the addition of organic solvent to the micellar solutions may reduce the fluorescence enhancements. When 2-propanol

was added to the micellar solutions of acyclovir, the fluorescence increase was lower, only two-fold. Separation on octadecylsilica with a mobile phase containing only SDS resulted in too high retention of acyclovir. A way of decreasing the retention was the addition of an organic modifier, but as commented, this had a negative influence on the fluorescence and could not be used. Therefore, the optimal conditions for the enhanced fluorimetric detection and for the separation may differ considerably, so that simultaneous optimization with respect to both detection and separation may be impossible.

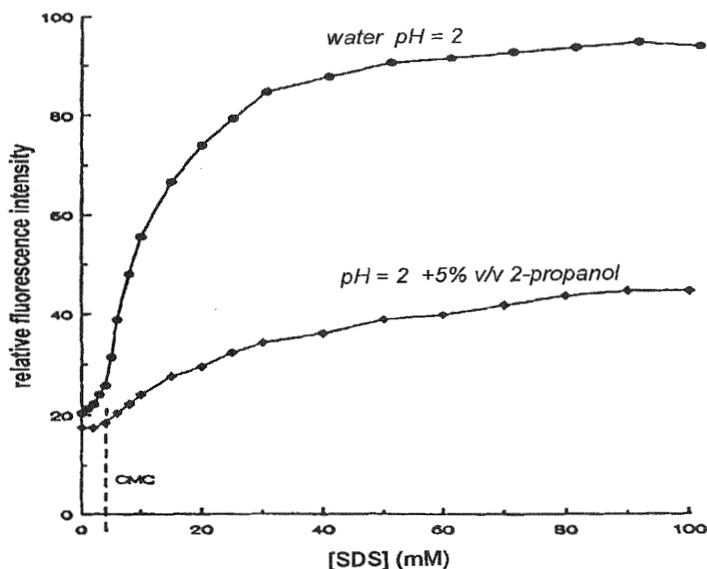


Figure 12.1 Relative intensity of fluorescence of acyclovir as a function of concentration of sodium dodecyl sulfate, without (●) and with (◆) addition of 5% 2-propanol, in a solution of 0.05 M phosphate at pH 2. Reprinted from Ref. 9.

There are several examples of significant improvements on the limits of detection (LODs) of compounds in MLC with fluorimetric detection, with respect to conventional Reversed-Phase Liquid Chromatography (RPLC). The LODs of a series of polycyclic aromatic hydrocarbons (PAHs) obtained in SDS micellar and methanol-water

eluent are compared in Table 12.2. The SDS mobile phase enhanced the fluorescence signal in a range from 1.5 for fluoranthene to 10 for pyrene.

Table 12.2 Comparison of LODs (ng/mL or ppb) for some PAHs obtained in MLC and Conventional RPLC with Fluorimetric Detection

Compound	Micellar Eluent	Aqueous-Organic Eluent
Acenaphthylene ^a	100	270
Anthracene ^b	0.2	0.2
1,2-Benzanthracene ^a	0.5	2.0
Benzo[<i>a</i>]fluorene ^a	270	480
Benzo[<i>e</i>]pyrene ^b	0.2	0.5
Biphenyl ^b	0.2	0.7
Fluoranthene ^b	2.5	3.8
Naphthalene ^b	0.3	1.2
Pyrene ^a	1.7	17.4
Pyrene ^b	0.25	2.6

^a Micellar eluent: 0.035 M SDS; aqueous-organic eluent: methanol-water 30:70 (v/v) [13].

^b Micellar eluent: 0.024 M SDS; aqueous-organic eluent: methanol-water 40:60 (v/v) [2].

Further enhancements in detection were obtained by the use of laser-induced fluorimetry with one of the visible lines of an argon ion laser (488 nm) for excitation [7]. The relevant properties of this source of radiation are its high intensity and excellent spatial resolution. A procedure was developed to determine several banned drugs in sports, such as acetazolamide, amphetamine, atenolol, chlorthalidone and furosemide, after separation with a 0.1 M SDS mobile phase. As the fluorescent drugs did not absorb at 488 nm, the highly fluorescent

fluoresceine isothiocyanate derivatives were formed before injection in the chromatographic column.

II.2. Analysis of Physiological Samples

The development of selective and sensitive analytical methodologies for the analysis of minute quantities of drugs, in physiological fluids, have attracted considerable interest in analytical toxicology and therapeutic drug monitoring. As shown in Chapter 11, MLC provides a solution to direct injection of physiological samples by solubilizing the protein components, via surfactant coating of the analytical column to avoid clogging. In addition, the surfactant monomers appear to displace the drug bound to the protein, releasing it for partitioning to the stationary phase. The possibility of direct sample introduction greatly simplifies the treatments and improves the accuracy of the procedures.

As commented above, most of the reported chromatographic procedures using micellar eluents utilized UV detection. This is not optimal for many drugs and samples. Fluorescence detection of compounds which yield measurable fluorescence emission may be preferable, owing to the higher sensitivity and selectivity when compared to absorption methods. In certain cases, the obtainable LODs will be significantly lower, more than adequate for therapeutic drug monitoring of concentration ranges normally encountered in serum and urine.

The fluorescence background signal of the physiological matrix at the solvent front, due to unretained proteins, is similar to that observed with UV detection (see Chapter 11). Again this is the limiting factor in the LODs, although in some cases, the signal seems to be substantially reduced. The background response level can be varied by changing the excitation wavelength. It has been found that serum background can be completely eliminated by using a 470 nm cutoff filter [6].

II.3. Detection of Metal Complexes

The determination of nonfluorescent analytes through reaction with suitable fluorescent reagents extends the number of compounds that can

be detected using micellar-enhanced fluorescence. An example is given by a highly sensitive and selective chromatographic-fluorimetric procedure for aluminum in human serum, after formation of the 8-quinolinol complex [11]. The interest in determining this metal is its relation with the pathology of Alzheimer disease and dialysis dementia.

The effect of micellar aggregates on the luminescence of organic compounds can be easily explained, whereas the introduction of a metal ion in these microheterogeneous systems significantly increases the difficulty to rationalize the observed effects. In fact, the mechanisms which are operating in micellar-enhanced fluorescence of metal complexes have not been completely elucidated. However, neutral complexes appear to be protected against deactivation pathways by the surrounding micellar environment [1].

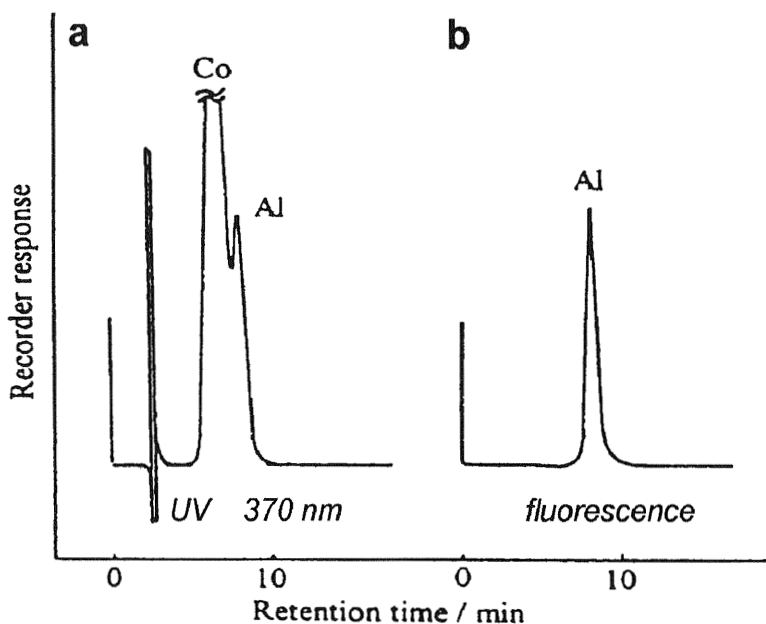


Figure 12.2 Chromatogram of 8-quinolinolato-metal complexes. (a) Absorbance at 370 nm, (b) fluorescence at $\lambda_{exc} = 370$ nm, $\lambda_{em} = 504$ nm. The sample contained 4×10^{-5} M Fe^{3+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Pb^{2+} and 4×10^{-6} M Al^{3+} . Reprinted from Ref. 11 with permission of Chemical Letters.

Among the common metal ions, only aluminum and cobalt gave peaks when complexed with 8-quinolinol and eluted with SDS-acetonitrile mobile phases. However, the peaks appeared very close to each other with spectrophotometric detection (Fig. 12.2). The selective determination of aluminum was only possible with fluorimetric detection. The addition of SDS as well as several other surfactants to the aluminum complex solution, increased the fluorescence intensity. The procedure did not require deproteinization prior to analysis. The most commonly used technique for aluminum in human serum is graphite-furnace atomic absorption spectrophotometry, which is often limited due to serum matrix interference.

III. SENSITIZED TERBIUM FLUORESCENCE

III.1. Detection of Anabolic Steroids

Anabolic steroids are generally used as therapeutic agents in clinical practice, but are also widely abused as performance enhancing drugs. The undesirable side effects ascribed to this practice have led to strict regulation or total prohibition in most countries. Analytical methodologies suitable for the determination of anabolic steroids in urine should meet two criteria:

- (i) LODs for free steroid should be below 1-10 ng/mL.
- (ii) Simultaneous determination of different steroids and their metabolites should be possible.

Except for a number of aromatic estrogens, the majority of steroids are nonfluorescent. Many steroids react with concentrated sulfuric and phosphoric acid to form fluorescent derivatives, but the lack of selectivity and the formation of a large number of uncharacterized products limit the application of this method in drug testing.

Alternatively, a fluorescent tag such as dansylhydrazine can be attached to the steroid molecule, but this again involves a lengthy derivation process and can yield extraneous fluorophores, precluding the method from routine analysis.

Because of these considerations, the development of a method for steroid determination that gives low LODs, but requires no derivation and little or no sample preparation is highly desirable. One viable alternative for steroid analysis in physiological fluids was given by coupling the sensitivity of sensitized lanthanide fluorescence in micellar media with the selectivity of MLC [12]. The advantage of the method lies in its speed and simplicity, while LODs are in the low ng/mL range.

III.2. Energy Transfer to Lanthanide Ions

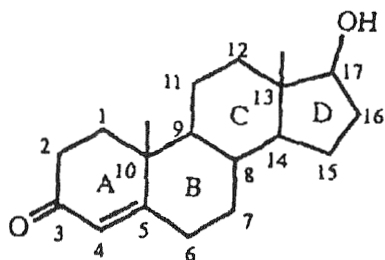


Figure 12.3 Structure of testosterone

The luminescence quantum yields of lanthanide ions, such as terbium and europium, have shown to be substantially increased when a highly absorbing sensitizer is used. Certain compounds having aromatic carbonyl groups can transfer energy to lanthanide ions in solution. Examples of such compounds are many anabolic steroids of abuse.

The testosterone derivatives possess a common structural feature, an

α,β -unsaturated carbonyl group in the A-ring, that also occurs in non-anabolics such as progesterone and cortisone (Fig. 12.3). For trivalent terbium ion to act as a good acceptor, the following conditions must be satisfied:

- (i) The excited energy level of the donor should be slightly above $26,000\text{ cm}^{-1}$ to minimize the energy difference with the excited singlet state of terbium.
- (ii) A nonaqueous environment should be maintained to prevent the strong quenching of terbium fluorescence by water.

- (iii) The donor and the acceptor should be less than $\sim 100 \text{ \AA}$ apart.

The first excited singlet level of a typical semiquinonoid steroid is at $41,666 \text{ cm}^{-1}$, corresponding to strong absorption at 240 nm (Fig. 12.4). The important feature for lanthanide sensitization is the existence of a triplet level at $26,041 \text{ cm}^{-1}$ which produces steroid phosphorescence that can be observed at 77 K . At room temperature, this triplet energy level can be used to efficiently pump the 5d_3 level of terbium ion, which occurs at $26,000 \text{ cm}^{-1}$. After relaxation to 5d_4 , this undergoes a radiative transition to the 7f ground level, resulting in the characteristic terbium ion fluorescence. Efficient energy transfer to lanthanide ion is made possible by the long lifetime of the excited donor. Fluorescence enhancements were also observed in trivalent europium, samarium and dysprosium fluorescence, but the effect was strongest with terbium, due to the close energy match between the donor triplet level and the terbium excited singlet state. Among the different steroids studied, bolasterone and testosterone acetate produced the greatest enhancements (approximately $\times 180$), with LODs in the 0.5 ng/mL range.

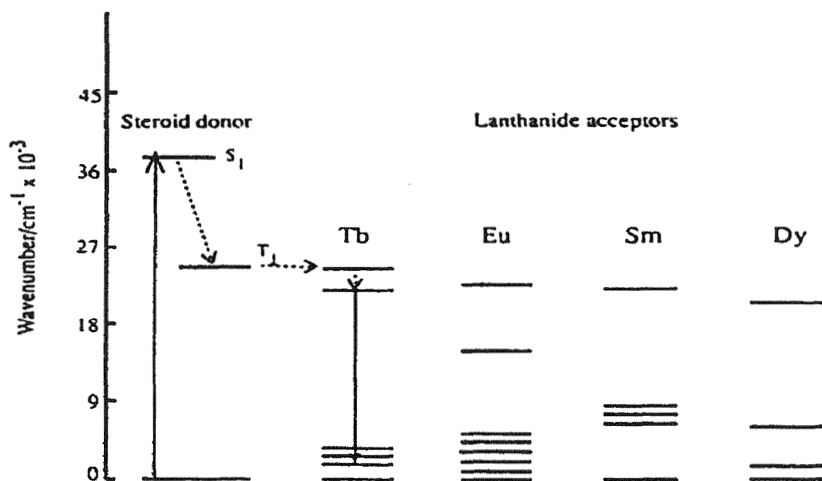


Figure 12.4 Energy level (Jablonski) diagram of typical semiquinonoid steroid donor and lanthanide ion acceptors. Reprinted from Ref. 12 with permission of the American Chemical Society.

Fluorescence was found to be quenched in physiological fluids such as urine. This problem was effectively overcome by putting together the terbium acceptor and the steroid donor in SDS micelles. A modest (50%) increase in terbium ion fluorescence was found at the cmc of SDS, which corresponds to the typical increase in lanthanide quantum yield when passing from water to an organic medium. This suggests that terbium was solubilized in the micelle, probably by accommodation in the palisade layer. The ion experiences shielding from the aqueous medium and is brought into enforced proximity to the donor also micellized. There should be however not only an effect of compartmentalization, the surfactant is also effective in separating excited steroid molecules from each other, reducing triplet-triplet annihilation.

The use of a micellar mobile phase simplifies the procedure by permitting the direct injection of urine into the column, without sample preparation. The micellar eluent contained 0.01 M $\text{Tb}(\text{NO}_3)_3$, 0.1 M SDS and 20% acetonitrile. A typical chromatogram showing the separation of testosterone, methyltestosterone, bolasterone, progesterone and testosterone acetate is shown in Fig. 12.5.

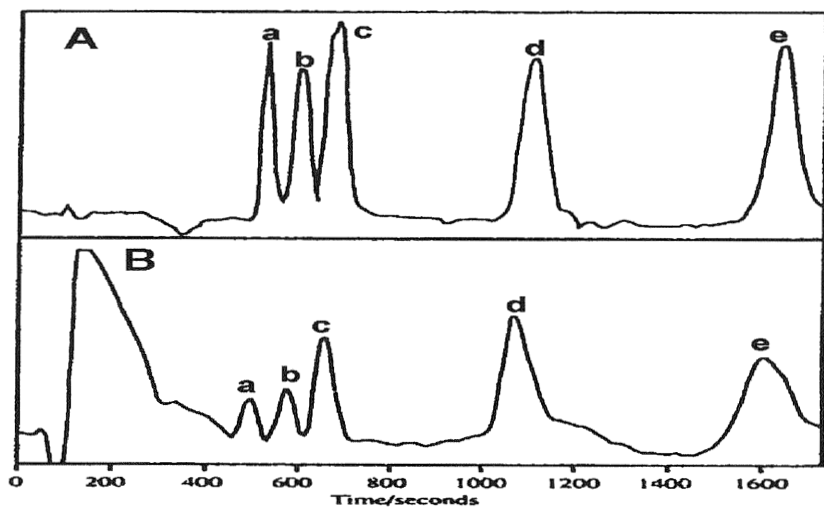


Figure 12.5 Steroid separation by MLC: (A) Aqueous solution with 20 ng of each steroid and (B) 200 μL of urine sample spiked with 300 ng/mL of testosterone (a) and methyltestosterone (b) and 100 ng/mL of bolasterone (c), progesterone (d) and testosterone acetate (e). Reprinted from Ref. 12 with permission of the American Chemical Society.

IV. MICELLE-STABILIZED ROOM-TEMPERATURE PHOSPHORESCENCE

IV.1. Requirements for the Observation of Phosphorescence

Molecular fluorescence has found extensive use in chemical analysis; in contrast, the utilization of phosphorescence has been severely restricted by the inconvenient and often time-consuming sample conditions required.

The phenomenon of phosphorescence is identified as the result of a radiative transition from an excited triplet state to the ground state. Because excited molecules tend to remain in the first excited triplet state, T_1 , for some characteristic long lifetime (ranging from ms to s), a high probability of nonradiative energy losses (quenching) exists, mainly by collisional deactivation. In order to observe phosphorescence, it is essential to minimize this nonradiative decay of the triplet state. The conventional approach has been to avoid collisions by freezing the sample to 77 K in liquid nitrogen to give a rigid frozen matrix (low-temperature phosphorescence) [22]. The reluctance of analytical chemists to use this technique arises from the need to cool samples to cryogenic temperatures, from tedious nonreproducible procedures and from limitations concerning solvent choice. An alternative to frozen glassy samples is stabilizing the triplet state at room temperature by adsorbing the organic compound on solid substrates, such as filter paper and surfaces used in thin-layer chromatography and drying the sample [22,23]. Room-temperature phosphorescence in fluid solution can be achieved by other techniques which use colloidal dispersions, cyclodextrins and micelles, that protect the rather fragile triplet excited state of some molecules. The technique using micelles has been called micelle-stabilized room temperature phosphorimetry (MS-RTP) [23].

Oxygen must be totally excluded from the sample and mobile phase, in order to observe MS-RTP. This is performed by bubbling purified nitrogen gas (passed through a secondary oxygen trap) through all solutions. The presence of a heavy atom (usually thallium or silver ions) as counterion is also required for generation of any appreciable

amount of phosphorescence. An exception is found with those compounds having internal heavy atoms, such as bromonaphthalene. Without the heavy atom, there is usually insufficient spin-orbit coupling which is necessary for intersystem crossing from the excited singlet state to the triplet state. Thus, the triplet state is not populated and phosphorescence cannot occur.

IV.2. Advantages of Micellar Liquid Chromatography in Phosphorescence Stabilization

Micellar solutions permit the observation of phosphorescence by using a conventional spectrofluorimeter and micro flow-cells. The advantage of this instrumental simplification was used to design a new detector in MLC [13]. Incorporation of a chromatographic column also proved to be quite advantageous for the stabilization of phosphorescence. Conventional static measurements of MS-RTP are labor intensive, subject to variability from incomplete and inconsistent removable oxygen from solution and require extensive sample purification. For good precision, it is necessary to illuminate the sample inside the apparatus for a fixed time (*e.g.*, 15 min), until the signal stabilizes.

The intrinsic precision and resolving power of MLC facilitate the routine observation of MS-RTP for three major reasons:

- (i) An in situ on-column purification allows the detection of pure solute, unencumbered by quenchers.
- (ii) The purification process renders it unnecessary to degas the individual samples; oxygen is not retained on the column and elutes on the solvent front undetected.
- (iii) The precise flow characteristics of the solvent delivery system enable each solute to pass through the detector flow-cell under consistent conditions, producing a solute illumination time which is constant from sample to sample.

As a result, relative standard deviations of the phosphorescence signal <2% have been routinely observed for replicate measurements in

MLC. Sample preparation/measurement time was also reduced from 45 min to <5 min [13].

All reports using MS-RTP detection employed SDS as surfactant [2, 13]. The requisite micellar reagent can be introduced into the system either as the mobile phase or as a postcolumn reagent. The surfactant concentration can therefore affect both the chromatography and the detector response. This stresses the importance of carefully separating the chromatographic and spectroscopic variables before comparing detection sensitivities. The presence of thallium ion used as heavy atom in the mobile phase did not significantly alter the elution behavior of compounds, compared to that in a pure SDS mobile phase [2].

MS-RTP detection is generally less sensitive than fluorescence. For many compounds, the percentage of fluorescence quenched by thallium is >90%, but not all of that quenching results in intersystem crossing. Also, the micelle itself, although effective, is not totally protective of the solute against radiationless pathways which results in luminescence intensity losses. The sensitivity of MS-RTP is still surprising considering that phosphorescence lifetimes are several orders of magnitude longer than fluorescence lifetimes, thereby permitting fewer excitation/emission cycles per unit time. In the first report on MS-RTP detection in MLC, written by Armstrong et al. [2], the authors expressed their concern on the relative weak phosphorescence intensity of several PAHs, compared to their fluorescence. Careful elimination of oxygen was made but a systematic weakness of the system, such as a small air leak, was presumed. In another report, linear dynamic ranges covering three orders of magnitude and LODs as low as 5 ng/L (5 ppb) were obtained for several PAHs [13].

The principal advantage of MS-RTP detection is the improved selectivity via detection of signals in the red region of the spectrum, which is inherently less crowded than the lower wavelength region. This is accomplished by red shifting the signal by intersystem crossing to the triplet state, which allows a higher wavelength cutoff filter to be used.

IV.3. Characterization of the Phosphorescence Signal

Conventional phosphorimeters can scan the spectrum of the sample to allow identification of the emission, based on energy/intensity profiles. This is not however possible with most chromatographic fluorescence detectors that use cutoff filters to pass a broad band of radiation. Additionally, in phosphorimeters, some sort of temporal discrimination is normally used to select an observation time window when the short-lived fluorescence and scatter has decayed to zero, but the long-lived phosphorescence is still present. When using a chromatographic flow-cell with no time delay available on the detector, it is necessary to devise another means of determining the nature of the observed signal. Ascribing a measured emission to fluorescence, phosphorescence, scattered light or a combination, requires careful verification.

Three approaches can be used to discriminate between the various types of signals. All rely on the fact that the excited triplet state of molecules is difficult to populate, especially for those with large fluorescence quantum yields and once achieved, it is very easy to deactivate by using radiationless processes. First, elimination of the heavy atom would virtually eliminate phosphorescence for most species. If a signal is still observed, it can be ascribed to fluorescence or scatter (except for molecules with a heavy atom substituent). Second, oxygenation of the mobile phase will introduce a quencher of the triplet state phosphorescence of the compound. If a signal exists in the presence of oxygen, it can also be safely designated as being fluorescence or scatter. These two methods of signal discrimination are illustrated in Fig. 12.6 for a mixture of 2-naphthol, biphenyl and phenanthrene. The chromatogram in Fig. 12.6A was obtained using a thallium free, oxygenated SDS mobile phase. The signals observed are due to fluorescence passed by a 418 nm lower cutoff emission filter in the detector.

Upon substitution of 30% of the sodium counterions in the SDS micelle with thallium, a similar chromatogram is observed (Fig. 12.6B). These signals also arise from fluorescence and are diminished in intensity compared to Fig. 12.6A, because thallium is effectively depopulating the excited singlet state from which fluorescence occurs. Thus, Fig. 12.6A

describes a sample in which virtually no triplet states are populated and Fig. 12.6B describes a sample in which the triplet state is appreciably populated but is radiationlessly quenched by oxygen.

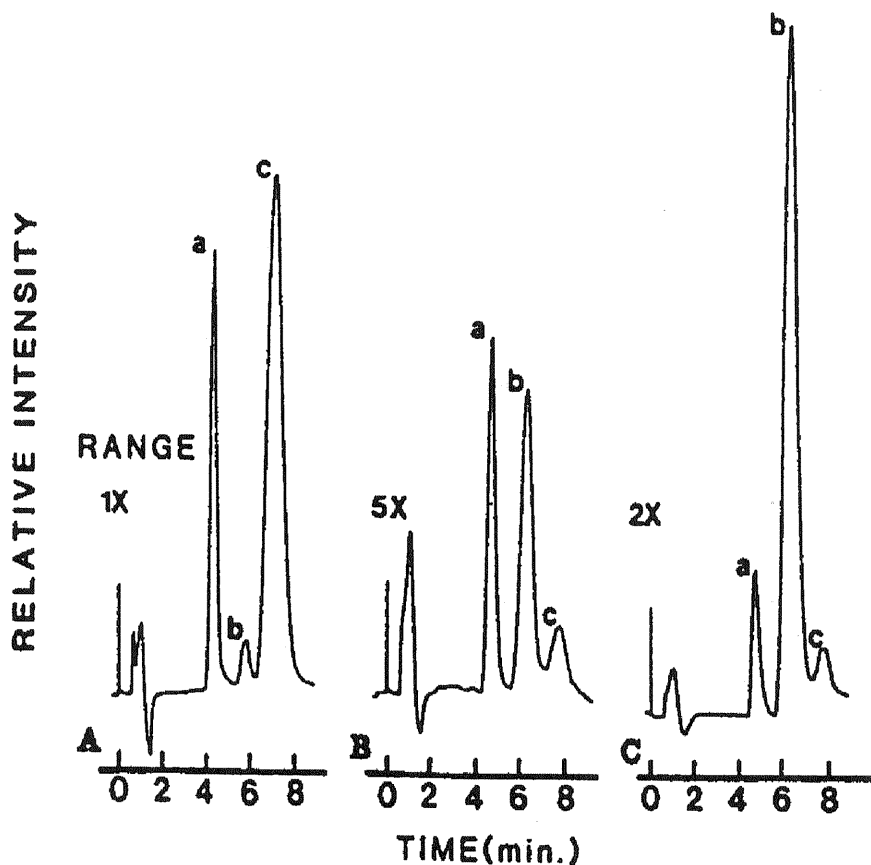


Figure 12.6 Chromatograms of 0.8 μg 2-naphthol (a), 16 μg biphenyl (b) and 0.4 μg phenanthrene (c), eluted from a cyano column with mobile phases of: (A) oxygenated 0.1 M SDS, (B) oxygenated 0.1 M SDS-Tl(III) dodecyl sulfate (70:30) and (C) oxygen-free version of (B). Full scale range expansions are indicated on each chromatogram. Reprinted from Ref. 13 with permission of the American Chemical Society.

Fig. 12.6C, obtained with an oxygen-free SDS micellar mobile phase with thallium ion, represents phosphorescence emission from the

three analytes and corresponds to the third approach. Some fluorescence may increase the signal, depending on the wavelength cutoff filter used. The phosphorescence response for biphenyl was enhanced 30- and 170-fold over 2-naphthol and phenanthrene, respectively, as compared to the relative response using fluorescence (Fig. 12.6A). The relative response enhancement for biphenyl could be increased further by using a longer wavelength cutoff filter which would discriminate more efficiently against unwanted signals.

V. INDUCTIVELY-COUPLED PLASMA-MASS SPECTROMETRY

V.1. Inductively Coupled Plasma-Mass Spectrometry as an HPLC Detector

Elemental speciation is becoming more and more important, since the environmental toxicity and biological importance of many elements depend on their oxidation states and different chemical forms. It is accepted today that the most reliable approaches for speciation are tandem techniques, such as hybrid analytical methods involving interfaced chromatography/atomic emission spectrometry (AES) or chromatography/inductively coupled plasma-mass spectrometry (ICP-MS). The low level detection capability of ICP-MS makes it especially attractive as an element-specific chromatographic detector in chromatography.

Unfortunately, common mobile phases for RPLC utilize organic solvents, which may be detrimental to the ICPs analytical performance. A decrease in sensitivity can result due to excessive solvent loading of the plasma. Higher plasma instability, increased background at certain masses due to the formation of molecular ions and carbon deposition on the sampling cone of ICP have been reported. Use of mobile phases that do not utilize the conventional organic solvents is therefore worthwhile. Micellar mobile phases have been proposed for RPLC/ICP-MS speciation

of alkyltin and arsenic compounds [14-16, 25]. The characteristics of these analytical procedures are commented on next. The preliminary optimization of the chromatographic separations was carried out with an ICP-AES detector, because of its ease of operation and accessibility (ICP-MS instrument time is often limited).

V.2. Speciation of Alkyltin Compounds

Organotin compounds are widely used as biocides, catalysts and polymer stabilizers. Very sensitive (subpart per billion detection) analytical methods are required to assess their effect on the environment. In addition, speciation information is necessary, since the toxicity of organotin compounds is strongly dependent on the number and nature of the organic substituents.

The feasibility of using MLC to separate organotin compounds with a C18 reversed-phase column was investigated [14]. Among the three types of surfactants (anionic SDS, cationic dodecyltrimethylammonium bromide, DTAB and nonionic Brij® 35), only SDS was found to resolve these compounds. The use of positively charged or nonionic micelle mobile phase resulted in a lack of interaction with the organotin cations. Therefore, these mobile phases caused the compounds either to come off with the void volume or to become irreversibly adsorbed on the stationary phase.

Salt deposition occurred at the constricted area of the torch injector when the SDS solution was introduced in the ICP-MS, with the regular torch positioned horizontally. This produced a significant diminution of the analytical signal with time. After approximately one hour, the injector was completely blocked. Several torch modifications were attempted, including a 2-mm-i.d. injector with a reduced taper and an injector with the taper region moved close to the injector inlet. A straight-tube injector with a 1.5-mm i.d. was also tried. Salt deposition still occurred. Finally, the deposition was eliminated by using a Leeman torch with a removable injector.

The clogging problem was further reduced by switching the column outlet to a 1% (v/v) nitric acid solution between chromatographic

runs. Nitric acid solution also rinsed the nebulizer, preventing it from clogging. With this interface, no deposition occurred over an 8 h continuous run. Addition of oxygen to the nebulizer gas was unnecessary as is usually the case with methanol-water or acetonitrile-water mobile phases. This reduced instrumental complexity and prolonged the life-time of the sampling cone.

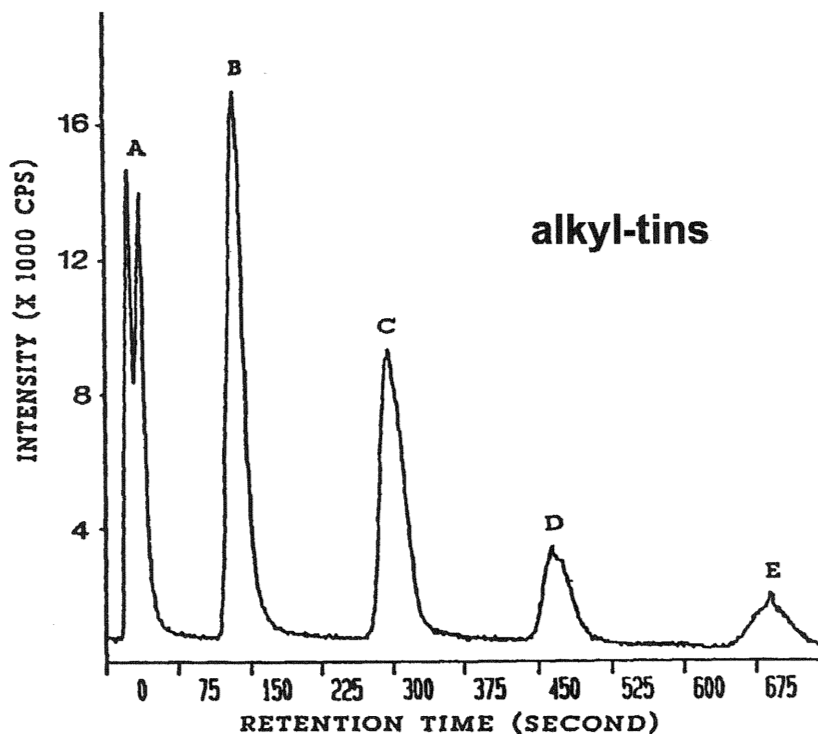


Figure 12.7 Chromatogram of a mixture of: (A) monomethyltin trichloride, (B) dimethyltin dichloride, (C) trimethyltin chloride, (D) triethyltin bromide and (E) tripropyltin chloride. The concentration of SDS was programmed from 0.02 M to 0.1 M for 2 min and constant at 0.1 M. Reprinted from Ref. 14 with permission of Applied Spectroscopy.

The concentration of SDS in the mobile phase was also kept to a minimum (*e.g.*, 0.1 M) to prevent clogging of the torch and sampling orifice on the ICP-MS detector. A good separation of organotin

compounds with short alkyl chains was obtained with this low concentration, but butyltin compounds showed too high retention. Fig. 12.7 shows the chromatogram of a mixture of alkyltin compounds. Peak splitting was observed for monomethyltin trichloride, presumably due to an equilibrium between different monomethyltin species containing different anions. Sub-mg/L level LODs were obtained for the five organotin compounds. Compared to an aqueous-organic mobile phase, MLC with ICP-MS detection provided better sensitivity.

V.3. Speciation of Arsenic Compounds

Arsenic has found wide use in pesticides, herbicides, wood preservatives and desiccants. Arsenic exposure can thus occur in many circumstances including occupations (*e.g.*, coal mines and smelters), air and food. The toxicity of arsenic varies widely with different chemical forms. As(III), As(V), monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA) are the arsenic species studied most in the literature. Inorganic arsenics are more toxic than organoarsenicals, while trivalent arsenic compounds are more toxic than their pentavalent counterparts. MMA and DMA are the two main metabolites known to be toxic.

The possibility of performing the direct injection of "dirty samples" such as physiological fluids, in MLC with ICP-MS detection, was studied. In aqueous solvents, the arsenic species dissociate to give anions. In this instance, the cationic cetyltrimethylammonium bromide (CTAB) became the surfactant of choice since it allows better partitioning of solutes into the micelles due to electrostatic interactions. For ICP operation, CTAB was kept below 0.05 M in the mobile phase to avoid deposition of salt at the nebulizer, spray chamber and torch injector tip. However, below 0.1 M CTAB, As(V) eluted at very long times (retention factor > 15) with very broad peaks, therefore, 1-propanol was added to the mobile phase to decrease its retention and improve the peak shape. Signal-to-noise ratios improved dramatically from 5% to 10% (v/v) 1-propanol, due to improved chromatography while having negligible degradation on plasma stability or analyte sensitivity.

Figure 12.8 shows a chromatogram of a mixture of DMA, As(III) and As(V). Total arsenic found was $0.52 \mu\text{g/mL}$ which was in agreement with the certified value of $0.48 \mu\text{g/mL}$. The last peak however was very broad and represented probably more than one arsenic species.

VI. AMPEROMETRIC DETECTION

VI.1. *Reduction of Adsorptive Fouling of Glassy Carbon Electrodes*

Electrochemical detectors are very popular in liquid chromatography. Electron transfer processes offer highly sensitive and selective methods for detection of solutes in flowing streams. Various techniques have been devised for these measurements, with the most popular being based on the

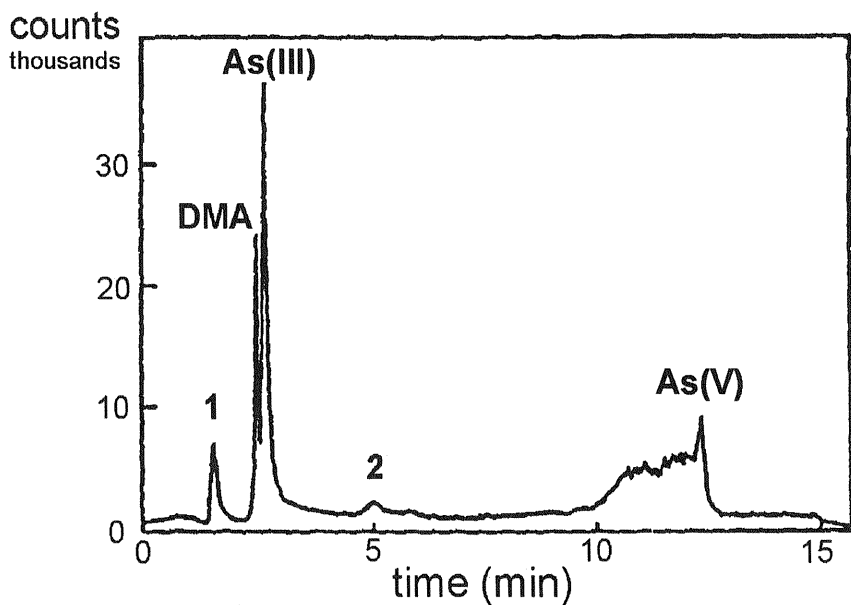


Figure 12.8 Arsenic speciation in urine. Peaks 1 and 2 are different forms of chlorine. DMA is dimethylarsenic acid. Column: Hamilton PRP-1. Mobile phase: 0.05 M CTAB-10% 1-propanol at pH 10.2. Reprinted from Ref. 16.

application of a fixed potential to a glassy carbon solid electrode. In spite of the wide use of this detection technique, one very serious problem exists. As amperometric detection is based on an interfacial rate process, it is inherently sensitive to surface contamination. Mercury drops, which periodically gives renewed surfaces, are difficult to use in flowing streams. Solid electrodes, which are better suited for these systems, are easily fouled. Many surface studies have been performed to better understand the surface reactions that occur and prevent the fouling process [26]. Coating of solid electrodes with polymeric materials has been reported to avoid the access to the electrode surface of large adsorbents, such as proteins. This approach is not applicable, however, to small analyte molecules which adsorb during or after the electron transfer process. Furthermore, the electroactive species must diffuse through the coated layer to reach the electrode surface and this may increase peak variance and have adverse effects on chromatographic resolution. While many other external treatments work well, they are time consuming as the cell must be dismantled and reassembled between treatments.

Kirchhoff et al. [27] investigated the factors influencing electroanalytical measurements in aqueous surfactant media and found that anionic SDS, cationic CTAB and nonionic Triton X-100 possess a wide potential window with low background currents within which electrochemical measurements can be conducted. While surfactants adsorb onto electrode surface, electron transfer can still occur between solute and electrode surface.

The use of mobile phases containing a surfactant to prevent the effects of adsorptive fouling of glassy carbon electrodes has been reported [28]. Aromatic compounds were utilized as probe solutes, the oxidation of which proceeds through a radical cation mechanism. Two hypotheses were considered by which surfactants might serve to reduce the adsorptive fouling of solid electrodes. First, micelles might solubilize the radical cations generated in the oxidation reactions and carry them past the electrode surface before adsorption can occur. In this sense, micelles of anionic surfactants would be best, as there will be both hydrophobic and electrostatic attraction of the radical cation to the micelle structure. Second, a cationic surfactant would adsorb onto the electrode surface and

electrostatically repel the radical cations, thus preventing the electrochemically generated products from depositing on the electrode surface.

Figure 12.9 shows three series of sequential injections of *p*-nitrophenol in an aqueous-organic mobile phase and micellar mobile phases of cetyltrimethylammonium chloride (CTAC) and SDS. The loss in response was virtually identical for the mobile phase with no surfactant and for the SDS micellar mobile phase, but the response for CTAC is approximately constant. Possibly, enough of the negatively charged SDS was adsorbed onto the electrode surface and attracted the radical cations. Meanwhile, the carrier stream containing the cationic surfactant prevented adsorptive fouling and allowed accurate, reproducible measurements of many repetitive samples before electrode cleaning would be necessary. Indeed, no loss of electrochemical response was observed after 55 sequential injections. As the preventive mechanism appeared to be a lack of adsorption of the surfactant onto the electrode surface and not any interaction with the micelles, sub-cmc concentrations of surfactant in the aqueous-organic mobile phases will probably show the same electrode stabilization.

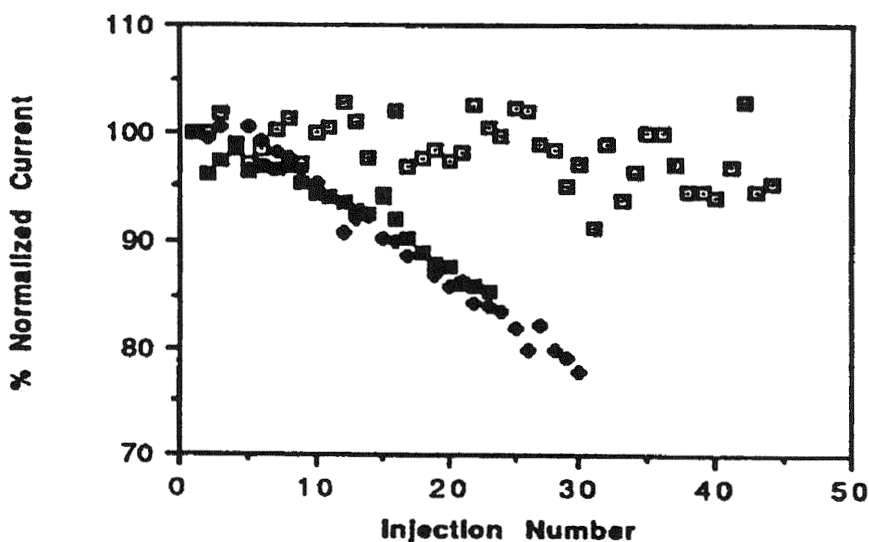


Figure 12.9 Normalized current vs. injection number for *p*-nitrophenol and various mobile phases: 0.10 M CTAC ($\square$), 0.10 M SDS ($\blacklozenge$) and acetonitrile-0.025 M phosphate 76:24 (v/v) ($\blacksquare$); pH was always 5.1. Applied potential: 1.1 V vs. Ag/AgCl. Reprinted from Ref. 28.

The adsorbed surfactant can change the double-layer structure, the rate of electron transfer and the apparent half-wave potential, $E_{1/2}$, of an electroactive species. Thus, it is clear that electrochemical conditions developed for an aqueous-organic separation are probably not directly transferable to micellar mobile phases. New hydrodynamic voltammograms will be necessary to determine the optimum operating potential. Figure 12.10 shows cyclic voltammograms of dopamine in 0.05 M HCl and 0.1 M SDS micellar solution. In micellar solution, dopamine is oxidized at lower operating potentials which should be a benefit for selectivity. Also the oxidizing current and the rate of molecular diffusion towards the electrode are lower, the latter produced by the higher local viscosity.

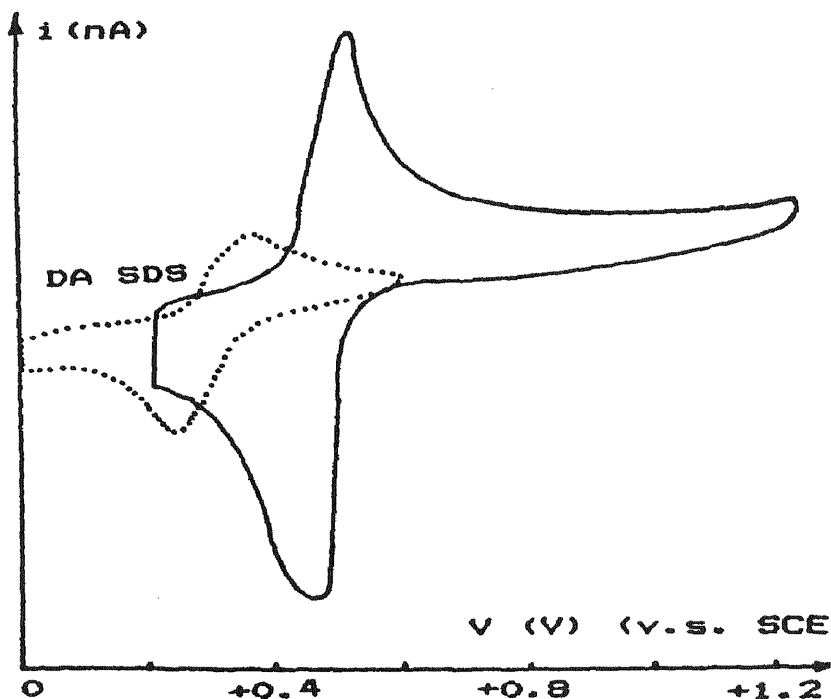


Figure 12.10 Cyclic voltammograms of dopamine obtained with 0.05 M HCl (solid line) and 0.1 M SDS (dotted line) vs. SCE. Reprinted from Ref. 18.

Microelectrodes have found increasing application in continuous-flow analysis. Their advantages include high current density, substantially reduced flow noise and negligible iR drop and capacitive charging current. A carbon fiber microelectrode detector was designed and used for the MLC assay of acetaminophen, an important analgesic drug [20]. The microelectrode device worked on the principle of the wall-jet cell, since it was fixed on a polyester sheet, the solution from the jet impinged perpendicularly on the microelectrode and spread radially over the surface of the sheet. As a consequence, only fresh species from the bulk solution reached the electrode. SDS was the surfactant of choice as it enhanced the electrochemical oxidation of acetaminophen. The sensitivity was higher than in traditional RPLC-electrochemical detection with glassy carbon electrodes.

VI.2. Compatibility of Micellar Gradient Elution with Electrochemical Detection

One of the major drawbacks of electrochemical detectors has been their limited compatibility with gradient elution, which is interesting for routine repetitive applications, in terms of time and solvent savings. The existing problem is based on the fact that the background current is dependent on solvent and electrolyte composition, so any change in mobile phase composition would shift the baseline. The gradient-induced baseline shift should be decreased to an acceptable value by adjusting the contributing factors.

In amperometric detection, the application of a potential to the working electrode generates a large transient or charging current, which decays rapidly. The steady-state background current is composed of two components. The first component results from electrochemical processes associated with the electrode surface. For glassy carbon electrodes, these processes extend from the oxidation of functional groups on the electrode surface and the formation of oxide layer(s) whenever oxygen evolution occurs, to the dissolution of adsorbed species and oxide layer, depending on the history of the solid electrode. The second component is a result of electrolysis of traces of electroactive impurities in the solution and/or oxidation of the solvent itself. Thus, the magnitude of the residual current

is dependent on the surface condition of the solid electrode and the rate of impurity and solvent oxidation.

The variation of mobile phase composition during gradient elution can alter the extent of the electrolysis of the mobile phase and impurities, thus resulting in a change of the residual current which contributes to the baseline shift. Also, changes in the double layer of the electrode induces a charging current flow (at least transiently). In general, changes in the properties of the mobile phase, such as pH, viscosity, electroactive impurities, ionic strength and solvent type, along other parameters such as temperature, flow-rate, cell resistance, potential and electrode sensitivity, influence the baseline shift [17, 29].

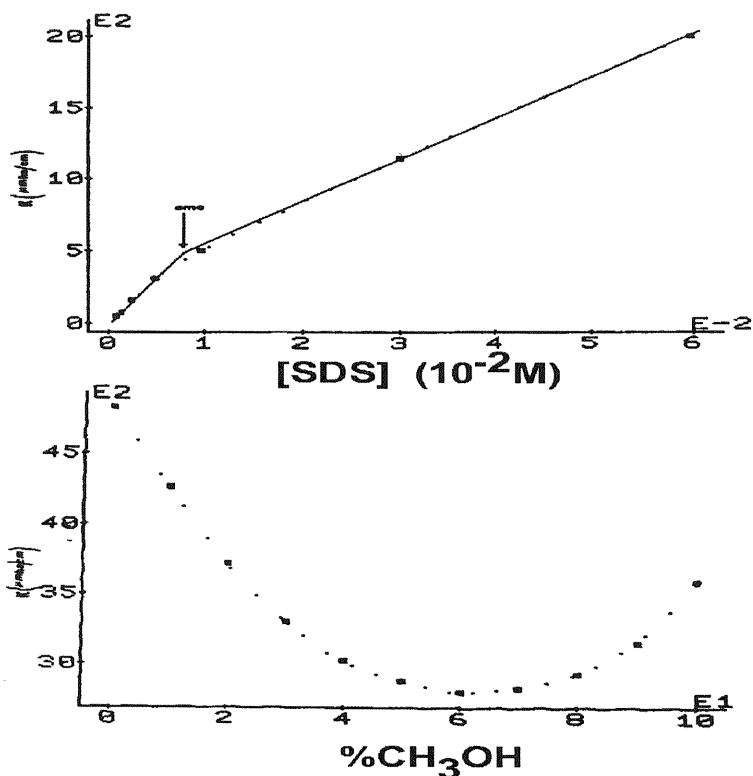


Figure 12.11 Specific conductance vs. micellar SDS concentration (top) and percentage of methanol in a methanol-water mixture (0.05 M NaClO₄) (bottom). Reprinted from Ref. 17 with permission of the American Chemical Society.

Dorsey et al. [17, 30] demonstrated advantages associated with the use of a micellar concentration gradient in MLC. Conventional RPLC with gradient elution requires a long column reequilibration time before the next injection. However, in MLC, only the mixer and injector must be flushed prior to the next injection. This is possible because any change in total surfactant concentration changes only the micelle concentration. The concentration of free surfactant remains approximately constant and since only free surfactant interacts with the stationary phase, minimal column equilibration is necessary. This fact suggested that micellar mobile phases might offer advantages for gradient elution with electrochemical detection. Due to the approximately constant concentration of free surfactant, it is likely that the double-layer structure and electrode surface conditions will also remain virtually unchanged during the course of a micelle gradient, reducing any possible contribution to the baseline shift from these factors.

Constant bulk solvent composition during a micelle gradient makes control of parameters such as pH, conductance and even mobile phase impurities, easier compared to aqueous-organic mixtures. Since water is the major constituent in the solvent, aqueous buffers can be used to balance the pH even in the presence of a small but fixed percentage of organic modifier. The conductance change of a micelle gradient is shown in Fig. 12.11. Since the conductance of micellar solutions is directly proportional to the concentration of ionic surfactant, this change can be minimized by adding more supporting electrolyte to the solution of lower micelle concentration. This cannot be achieved in aqueous-organic gradients as the conductivity of methanol-water mixtures passes through a minimum during the gradient.

A range of gradient concentration from 0.01 M SDS (just above the cmc) to 0.40 M (wider than necessary for many practical applications) was checked to give good results. Figure 12.12 shows the chromatogram of a mixture of eight phenols. This figure is a demonstration of the slight gradient-induced baseline shift under controlled conditions. Compatibility of gradient elution techniques with MLC is determined by both chromatographic and electrochemical conditions.

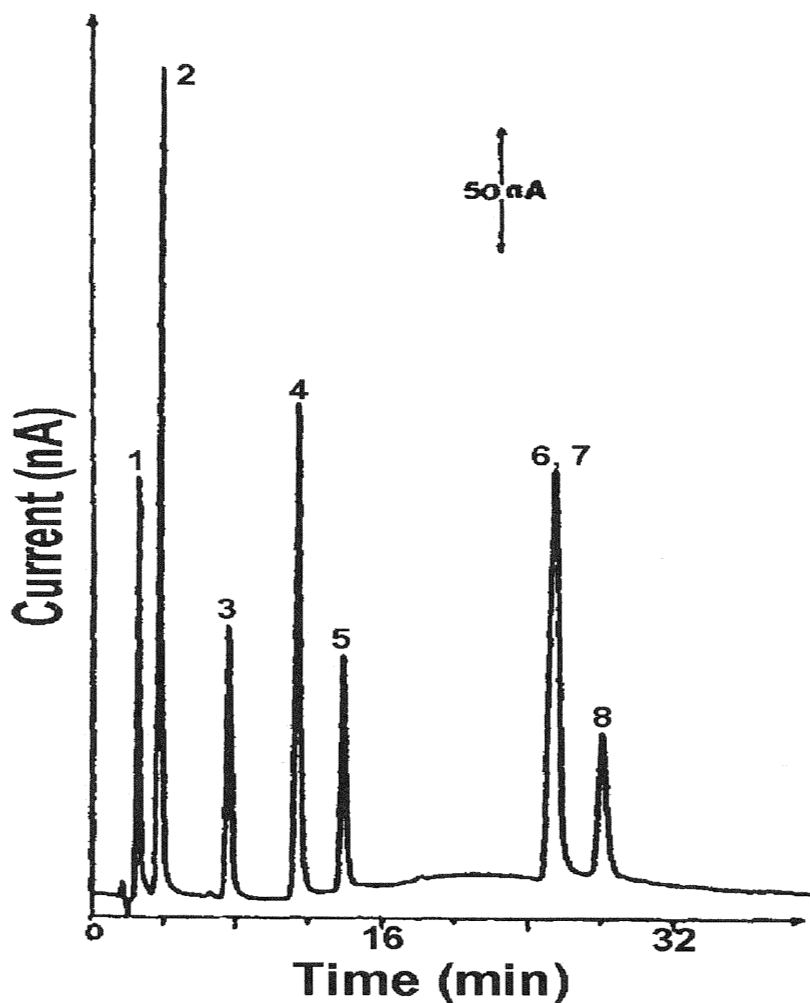


Figure 12.12 Micelle gradient chromatogram of phenols (ca. 400 ng of each) with detection at 1.20 V vs. Ag/AgCl. Solvent A: 0.05 M SDS-3% 1-propanol at pH 2.5 (phosphate buffer). Solvent B: 0.112 M SDS-3% 1-propanol at pH 2.5. NaClO_4 was added to balance conductivity with solvent B. Gradient program: Solvent A to B in 15 min. Compounds: (1) hydroquinone, (2) resorcinol, (3) catechol, (4) phenol, (5) *p*-nitrophenol, (6) *o*-nitrophenol, (7) *p*-chlorophenol and (8) *p*-bromophenol. Reprinted from Ref. 17 with permission of the American Chemical Society.

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13

Expanding the Micellar Liquid Chromatography Field

I. INTRODUCTION

MLC uses micellar mobile phases with classical RPLC columns. This chapter expands the field to include some mobile phases that can be considered close to micellar phases, such as normal and reverse micro-emulsions, bile salt solutions, and surfactant solutions in supercritical fluids. Also, this chapter rapidly surveys the use of micellar mobile phases with non-RPLC stationary phases such as size exclusion or gel permeation polymer phases. Allied techniques using micellar phases such as ion-exchange chromatography and capillary electrophoresis are also briefly presented.

Surfactant enhanced chemical separations are obtained through coacervation, liquid membranes, ultrafiltration, foams and/or other interactions with phospholipids, proteins and biomolecules. All these topics were deliberately excluded. They are well exposed in the literature [1-3].

II. USE OF ORIGINAL MOBILE PHASES

Chapter 2 discussed microemulsion structure. These organized media are stable and transparent. They are possible candidates for mobile phases in chromatography. Bile salt solutions are another kind of special micelles with chiral properties that can be used in MLC as well. Supercritical fluids (SF) were also used as surfactant solvents to perform micellar SFC, a variation of MLC.

II.1. Oil in Water Microemulsions

a) Physicochemical Structure

The effect of the addition of short chain alcohols on the chromatographic selectivity and peak efficiency was extensively exposed in previous chapters. The addition of such alcohols to a micellar solution forms mixed micelles. This is the first step toward the achievement of microemulsions with ionic surfactants. The oil in water microemulsion (L1 structure, see Chapter 2) has a continuous aqueous phase containing oil swollen micelles or microdroplets of oil stabilized by an alcohol-surfactant interphase layer. The medium is transparent and stable, however it has a dynamic structure. Then, it is interesting to see if L1 microemulsion mobile phases could be useful in MLC.

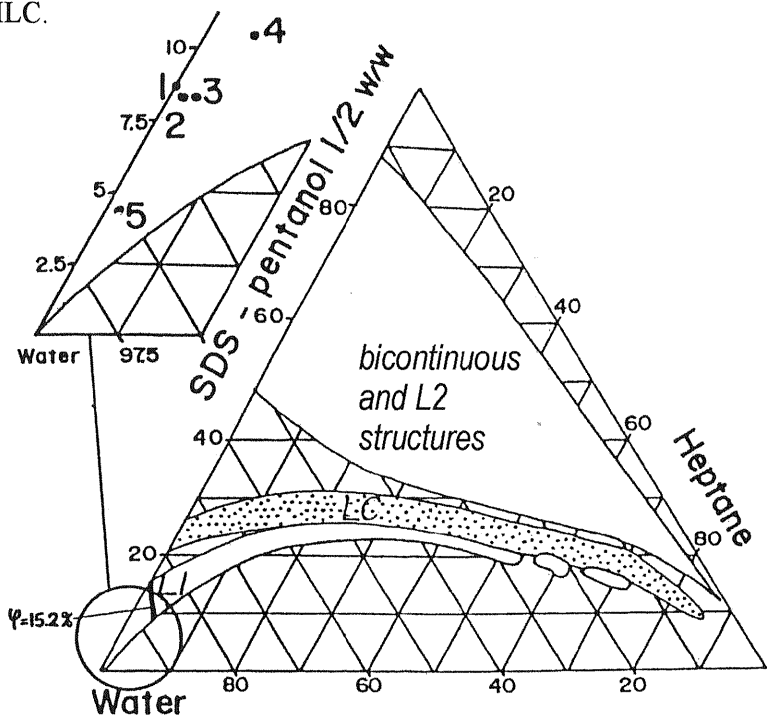


Figure 13.1 Mass phase diagram of the system water-heptane-SDS-pentanol (ratio 1/2 w/w). The open areas show the clear microemulsion compositions. The circle focuses on MLC useful compositions. The corresponding inset figure shows the numbered microemulsion whose compositions are listed in Table 13.1. Reprinted from Ref. 4.

b) *The System*

Figure 13.1 shows a microemulsion system that was extensively studied in MLC [4, 5]. Appendix IV at the end of the book describes the experimental protocol that allows to obtain the mass phase diagram of a microemulsion system not found in the literature. The phase diagram of the heptane-water-pentanol/SDS system (Figure 13.1) was established using this protocol. Only compositions located in the water-rich corner of the diagram were tested. The ϕ parameter, in v/v percentage, corresponds to the nonaqueous microemulsion content (oil + alcohol + surfactant). The high viscosity of the o/w microemulsion systems with less than about 85% w/w water ($\phi > \sim 20$) precluded their use in LC.

Table 13.1 Mobile Phases Microemulsion Compositions Corresponding to Fig. 13.1

Micro-emulsion #	SDS % w/w	Pentanol % w/w	Heptane % w/w	Water % w/w	ϕ % v/v
1	2.88	5.76	0	91.36	8.64
2	2.73	5.51	0.31	91.45	9.21
3	2.75	5.51	0.59	91.15	9.60
4	3.52	7.00	1.44	88.04	13.37
5	1.44	2.87	0.34	95.35	5.09

ϕ is the organic volume fraction, $1 - \phi$ is the aqueous volume fraction. Data from [4].

c) *Microemulsions for the Rapid Screening of Illegal Drugs in Sports*

Oil in water microemulsions were first used as mobile phases in MLC to solve a practical problem: the screening of drugs illegally used in sports [4]. Eleven drugs were separated on a 25×0.46 cm C18 5 μ m column (Spheri 5, Brownlee Labs) with the five microemulsion mobile phases whose compositions are listed in Table 13.1 and represented by a dot in Fig. 13.1.

b) *The System*

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The elution strength and selectivity obtained with the o/w microemulsion mobile phases was acceptable. For example, the k factor of the apolar acebutolol and nadolol compounds (β adrenergic blockers) were 13.5 and 18.2, respectively, with a 100% methanol mobile phase (selectivity $\alpha = 1.5$). The corresponding retention factors were 14 and 3 with Microemulsion #2 ($\alpha = 4.7$) and 5.5 and 1.8, respectively, with Microemulsion #4 ($\alpha = 3.1$) [4]. Chlorthalidone, a polar diuretic, was almost not retained with the 100% methanol mobile phase ($k = 0.37$). With Microemulsions #3, #4 and #5, its retention factors were 1.04, 0.76 and 1.83, respectively. Figure 13.2 shows that it was possible to directly inject urine samples. Proteins and/or UV absorbing biological material produced an early eluting peak whose width was related to the microemulsion heptane content [4].

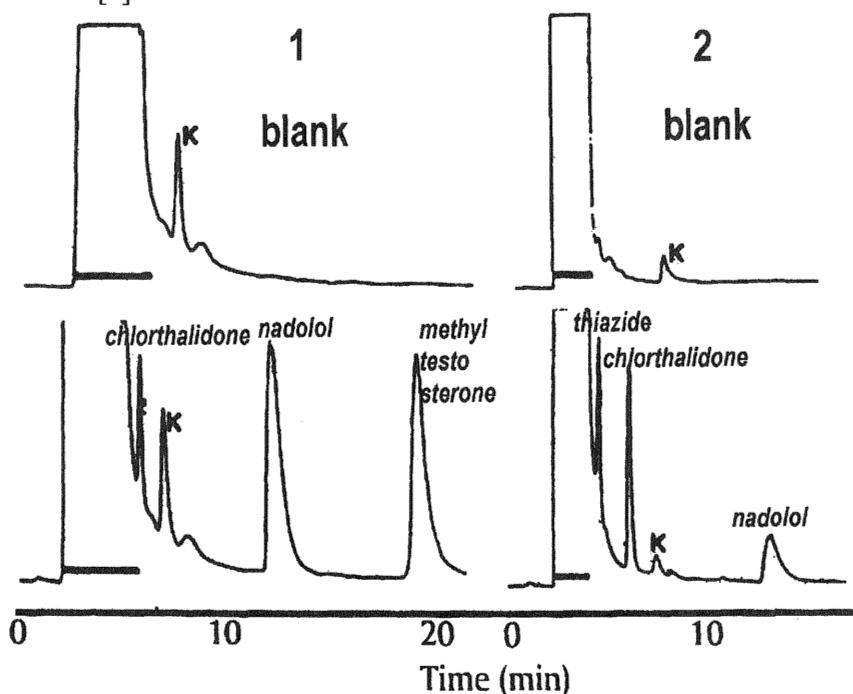


Figure 13.2 Direct injection of a urine sample after 50% dilution with the microemulsion mobile phase. Column: 25 cm $\times$ 4.6 mm i.d., 5 μ m C18 Spheri 5, flow rate 0.5 mL/min, 10 μ L injection, detection UV@254 nm. Left chromatograms: Microemulsion #1 (Table 13.1) pressure drop 184 kg/cm²; right chromatograms: Microemulsion #2, pressure 160 kg/cm². Reprinted from Ref. 4.

Two points were noticed. The first point concerned selectivity. The drug retention could be modeled using the very simple relationship:

$$k = a \log \varphi + b. \quad (13.1)$$

The classical $1/k$ vs. φ relationship was poorly linear. All the k vs. $\log \varphi$ lines were crossing together close to the coordinate range: $\log \varphi = 1.18 \pm 0.04$ and $k = 0.5 \pm 0.05$ [4]. The second point concerned peak efficiency. A dramatic 3-time decrease of plate count was noticed between microemulsion #1, #2 and #3 when the k factors were reduced by 10 to 30%. To find the reasons for these observations, a complete study was done with the alkylbenzene homologous series.

d) Behavior of the Homologous Alkylbenzene Series

Sixteen different water-rich microemulsion compositions from Fig. 13.1 were tested as mobile phases on a 15×0.46 cm Spherisorb $5\mu\text{m}$ C18 column (SFCC, France). The alkylbenzene homologous series, from toluene to decylbenzene, was used as the test solute [5].

Selectivity. A linear relationship between the retention factors and the alkyl chain carbon number, n_c , was obtained with all 16 microemulsion mobile phases:

$$k = \alpha n_c + \beta \quad (13.2)$$

The regression coefficients were higher than 0.984. Such linear relationships were previously obtained with micellar mobile phases as detailed in Chapters 7 and 8. The slopes, α , and intercepts, β , of these lines were found to be related to the organic volume fraction, φ , of the microemulsion:

$$\alpha = 10^{e\varphi + f} \text{ and } \beta = 10^{g\varphi + h} \quad (13.3)$$

The e, f, g and h constants were determined so that the general empirical expression for the alkylbenzene retention factor with such microemulsion mobile phases could be expressed as:

$$k = 10^{-0.154\phi + 1.43} n_c + 10^{-0.077\phi + 1.73} \quad (13.4)$$

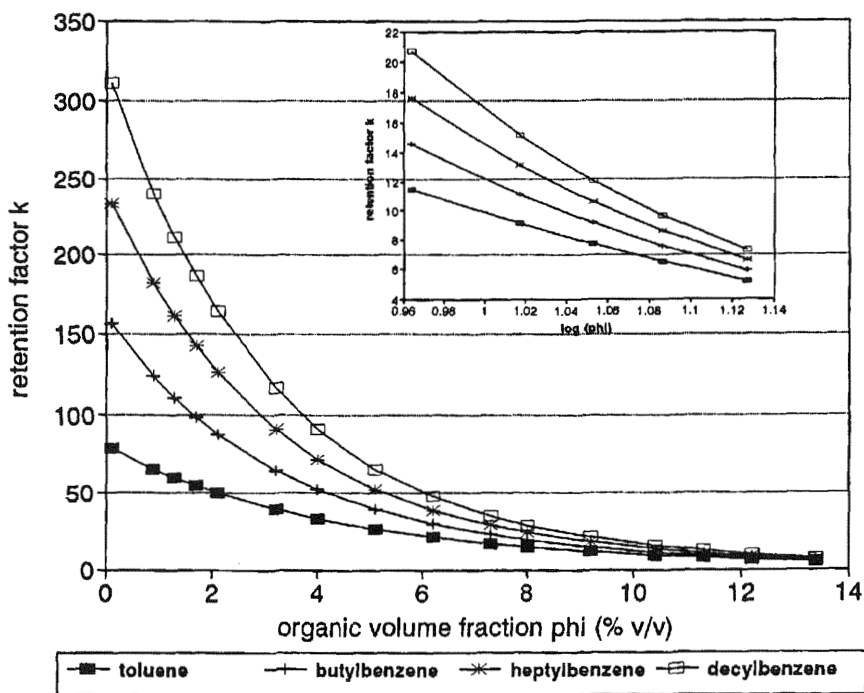


Figure 13.3 Retention factor of four alkylbenzenes versus the organic volume fraction, ϕ , of the microemulsion mobile phase. Inset: The same data plotted versus $\log \phi$ seem linear.

Figure 13.3 shows the k vs. ϕ curves. The inset shows the k vs. $\log \phi$ curves for the composition with $\log \phi$ ranging between 0.70 and 1.12 ($5\% < \phi < 13\%$). These curves seem linear. A regression analysis done on these points only returned the slopes and intercepts of the straight lines with

regression coefficients in the 0.981-0.995 range. Furthermore, all the lines crossed together around the coordinate range $\log \varphi \approx 1.15$ ($\varphi \approx 14\%$) and $k \approx 5$. Of course this result is an artifact with no chemical meaning. It shows that one should be careful of such possible mathematical artifacts when analyzing any set of experimental data with powerful modern software. The only significant result is that organic rich microemulsions have a strong solvent power. The retention factors of all members of the alkylbenzene family become similar with a nil selectivity.

Efficiency. It has been shown that an increase of the microemulsion pentanol content can increase the efficiency [5]. Conversely, a drastic decrease of efficiency has been observed upon increasing the heptane content in the microemulsion [5]. The toluene efficiency dropped from 7000 plates with Microemulsion #1 (without heptane) to 3300 plates with Microemulsion #3 (heptane 0.59% w/w). The decylbenzene peak efficiency was divided by 8 with the same addition of heptane (1150 plates and 140 plates, respectively) [5]. It was speculated that the significant decrease in the kinetics of the solute exchange between the stationary phase and the microemulsion droplets was linked to physicochemical structural changes of the system [5]. The large solubility power of o/w microemulsion systems, up to 4 g/L of decylbenzene in a 90% water rich system, produced a unique selectivity. Unfortunately the low-efficiency problem hinders the use of such systems.

II.2. Water in Oil Microemulsion

The use of water in oil microemulsions (L2 type) was also termed "normal phase MLC." Dorsey was the first to use such reversed micellar mobile phases with two polar silica stationary phases, an unbonded and NH_2 bonded phase [6]. His goal was to suppress the retention and selectivity variations caused by the water content of apolar solvents. It was shown that the retention of phenol, naphthol and dinitrotoluene was not sensitive to the water content (range 0.1-1% v/v) of an AOT-hexane mobile phase. In this concentration range, water molecules are bound tightly to the ions (AOT sulfonate polar heads and sodium counterions) with practically no free water molecules left to adsorb on the polar stationary phase.

When large amounts of water (2 to 40% w/w) are added to hexane or heptane-AOT solution, the water/AOT molar ratio, $M_{W/AOT}$, becomes the important parameter [7].

Selectivity. The retention factors of test solutes were dependant on the $M_{W/AOT}$ molar ratio and the heptane content. Fig. 13.4 shows that the k variations were not monotonous. It was demonstrated that the hydration of the polar unbonded silica surface changed significantly in the $0 < M_{W/AOT} < 10$ range. The AOT surfactant and water adsorbed on the silica surface forming a composite layer so thick that it changed the column permeability and dead volume [7]. The combined effect of stationary phase hydration and adsorbed layer was responsible for the observed retention factor variations (Figure 13.4). The $M_{W/AOT}$ ratio is directly related to the water droplet size so the retention factor of a test solute can be related to the structure of the L2 microemulsion.

Efficiency. The peak efficiencies obtained with the L2 microemulsion mobile phases for various solutes were compared to the ones obtained with classical hexane-2-propanol phases. A 2- to 3-fold lower plate count was obtained with the L2 microemulsion mobile phases compared to the traditional mobile phases [6]. In another work, the efficiency obtained with the heptane-AOT-water microemulsion was in the 200 plate range, 10 times lower than the values obtained for the same solutes and the same column with traditional heptane-propanol phases [7]. Once again, the exchange of the solute between the stationary phase and the reversed micelles is slow. The efficiency problem in the case of microemulsion mobile phases is so serious that the practical use of these mobile phases in chemical analysis is questionable [8]. For physicochemical research such as microemulsion structure investigation, MLC can help.

II.3. *Supercritical Micellar Mobile Phases*

a) *Physicochemical Structure*

The supercritical state of pure compounds exists at elevated temperature and pressure. Above a compound-specific critical temperature and critical pressure, the liquid state and gas state disappear, replaced by a unique supercritical state. Supercritical fluids associate some properties of gas (low

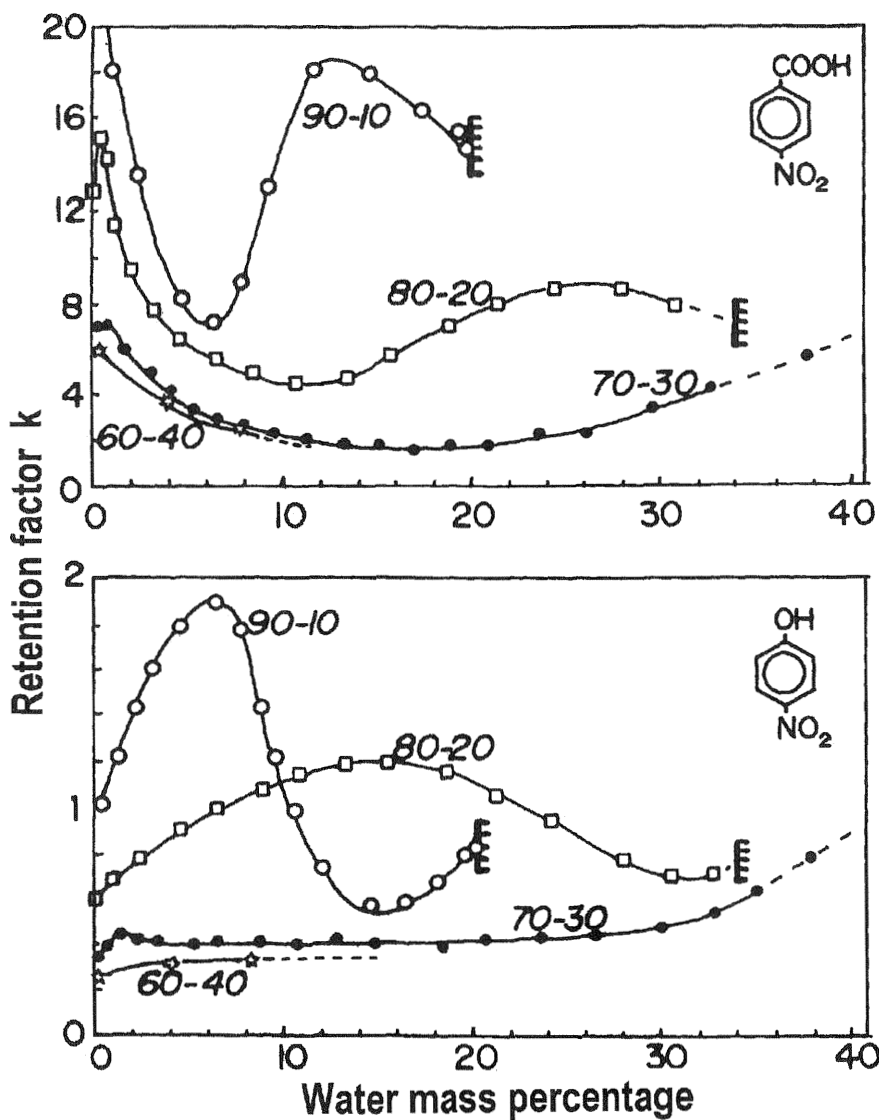


Figure 13.4 Retention factor of polar solutes with L2 W/O microemulsion mobile phases. Column: 15 cm $\times$ 4 mm i.d., 10 μ m XWP 250 Å Grace bare silica, flow rate 0.5 mL/min. Reprinted from Ref. 7 with permission of the American Chemical Society.

viscosity, high compressibility) to some properties of liquids (density, solvent power). This last property makes them useful in separation and extraction. The solvent power of a supercritical fluid depends on its density, i.e. it is adjustable in changing temperature or pressure. Carbon dioxide is the most commonly used supercritical fluid. Its critical parameters are 31.0°C (304.1 K) and 7.38 MPa (73.8 bars, 1060 p.s.i.). Propane can also be used ($T_c = 97^\circ\text{C}$ or 370K; $P_c = 4.33$ MPa or 43.3 bars or 620 p.s.i.), as well as nitrous oxide, ethane, methanol or even water. The diffusion coefficient of solutes dissolved in such phases is intermediate between the corresponding gas and liquid values. Supercritical phases, CO_2 or propane, but even water, are rather apolar phases. The idea was to adjust their solvent power using surfactants in the reverse micelle form. Only supercritical ethane and propane were able to solubilize the AOT surfactant and some water in reversed micelles [9]. Is it possible to use such phases in chromatography?

b) Micellar SFC

There are several appealing factors for the use of micellar supercritical phases in chromatography. The peak efficiencies obtained in SFC are higher than in LC because the solute diffusion coefficients are higher in supercritical fluids than in liquids. In SFC, mass transfers are enhanced by the combination of high diffusion coefficients and low viscosities. This could compensate for the low efficiency induced by micelles. The polar aqueous core of the reverse micelles should allow the separation of hydrophilic or even ionic solutes with supercritical fluids. These polar compounds are difficult to analyze in SFC [10].

Unfortunately, the use of reverse micelles in supercritical propane was somewhat disappointing. Phenol, naphthol and resorcinol were separated using a 0.05 M AOT–0.25 M water reverse micellar phase in supercritical propane. However the efficiencies obtained were average, in the 5000 plate range ($h = 10 d_p$) for a 250×1 mm $5\mu\text{m}$ column [9]. Neither the FID universal detector nor the mass spectrometer could be used due to the AOT molecules. The adjustment of the AOT/water ratio required a complex pumping arrangement. SFC as a separation technique is used less frequently. Micellar SFC would need more work to fully understand the physical and chemical processes involved in the separation of polar and apolar solutes. At the moment, this path seems to be set aside.

II.4. Micellar Bile Salt Mobile Phases

a) Bile Salt Description and Properties

Bile salts are biosurfactants naturally formed by cholesterol degradation in the liver. Bile acids are stored in the gall-bladder. After derivation with glycine or tauric acid, the sodium salts of the derivatives are essential in the digestion process to emulsify food triglycerides. The pancreatic lipase enzyme can only split emulsified fatty globules.

The three main bile acids are cholic acid, lithocholic acid and deoxycholic acid.

R1	R2	name
OH	H	cholic
H	H	lithocholic
OH	H	deoxycholic

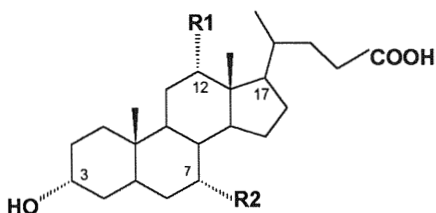


Figure 13.5 shows that the steroid skeleton of the molecule has a polar side and a lipophilic apolar side. Micelle formation is possible with a significant solubilization power for apolar molecules. The bile salt cmc depends on the pH and ionic strength. It is approximately 5-10 mM for cholic acid and 3-5 mM for the deoxycholic acid [11]. The great and appealing property of the bile salt molecules is their chiral structure with 3 or 4 asymmetric centers. Investigating micellar bile salt mobile phase in LC, it was hoped that their micelles would be able to distinguish enantiomers.

b) Micellar Bile Salts Mobile Phases

Hinze was the first to investigate the capabilities of micellar bile salt mobile phases [11, 12]. He found that a significant amount (~5% v/v) of a long chain *n*-alcohol (pentanol, hexanol or heptanol) was useful to minimize the bile salt adsorption on the C18 stationary phase. A wide range of solutes could be separated by these phases, PAHs, quinones, steroids, indoles, polar and lipophilic vitamins. These phases were also able to resolve optically big enantiomers such as binaphthyl derivatives [12]. Such compounds are

employed in organic synthesis schemes for enantioselective synthesis. The bile salt mobile phases were thoroughly evaluated for the enantioresolution of binaphthyl derivatives with different anionic, cationic, nonionic and even zwitterionic cholic acid derivatives [13-15]. Selectivity values as high as $\alpha=1.6$ were obtained for some binaphthyl derivatives. However, the mobile phase might not be a micellar phase since it contained 70% v/v acetonitrile [14]. Bile salt mobile phases were not able to separate enantiomers with simple asymmetric carbons.

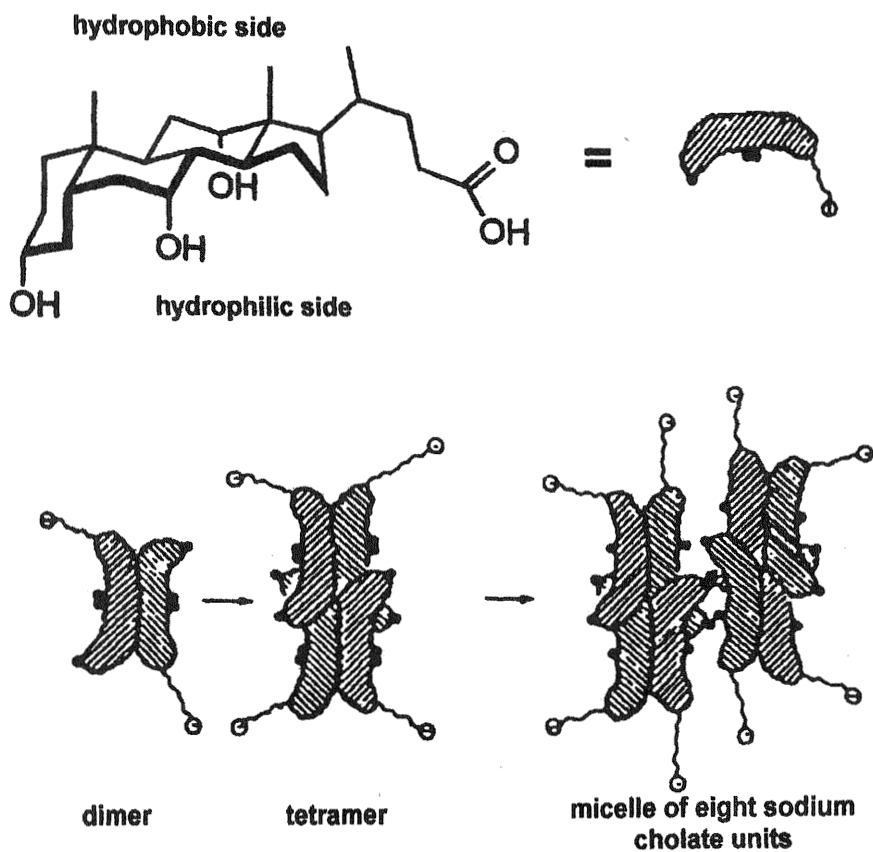


Figure 13.5 The cholic acid molecule and its aggregation pathway.

III. USE OF ORIGINAL STATIONARY PHASES

III.1. Micellar Gel Permeation Chromatography

Gel Permeation Chromatography (GPC) is also called Size Exclusion Chromatography (SEC). The principle is simple: big molecules or “objects” are sorted by size using some kind of stationary phase acting like a sieve. The exact mechanism is not actually a sieving process. The stationary phase contains pores of a given diameter. The molecules bigger than the pore size are not retained, they are “size excluded.” The smaller molecules visit the pores and need more time to pass through the column. The peak retention volume on the chromatogram corresponds to the molecule size, the bigger molecules eluting first, the smaller ones last. The molecule sorting size depends on the pore distribution of the polymer used as a stationary phase in the GPC column.

a) Separation of Small Molecules by GPC

In 1964, Herries thought of using micelles as the big “objects” not retained or excluded by a GPC polymer phase. Small molecules are retained by the pore of the stationary phase. They are less retained when solubilized inside an excluded micelle. The idea was to measure solute affinity for micelles through solute retention times. This was the first time a micellar phase was used in chromatography [16]. This part of MLC history was already exposed in Chapter 3. Terabe and Okada developed a slightly different approach to model the small molecule and ion retention in micellar GPC [17, 18]. The equation is:

$$\frac{V_i}{V_R - V_o} = \frac{1 + v(P_{WM} - 1)[M]}{K_D + P_{WS} \frac{V_s}{V_i}} \quad (13.5)$$

in which the subscripts i, R, o and s for the volumes, V, correspond to the internal pore, retention, void and packing polymer volume, respectively. The subscripts WM, WS and D for the distribution coefficients P or K are, respectively the solute micelle-aqueous phase coefficient, affinity (adsorption) of the solute for the polymer stationary phase and GPC

coefficient very close to unity for small solutes [17] ($K_D = 1$ for ions [18]). $[M]$ is the concentration of micelles. The plots of the left member of eq. 13.5 versus the micelle concentration were linear. Their slopes and intercepts allowed to obtain the P and K parameters for polar alcohols and phenols.

b) Ion Separation

Okada separated ions using ion-exchanges between ionic micelles and the aqueous phase. The principle is similar: the micelles are excluded from the pore volume of the polymer stationary phase; they travel faster than a small ion that visits all pores. When the ion is attached to a micelle, it also travels faster. Ion retention volumes allow to estimate ion micellar affinity. The surfactant-covered polymer stationary phase was also responsible for part of the ion retention [18, 19]. Equation 13.5 was used to determine the P coefficients of several inorganic anions with cationic micelles [18] and several metal-cations with anionic micelles [19]. Carboxylic anions were separated by mixed micelles made of SDS and Brij® 35 (C12E23) [20]. Several factors affect the selectivity in micellar GPC of inorganic anions [21]. The ionic strength and the amount of adsorbed surfactants were especially important. The pH and the micelle charge density also affected the ion separation. These factors indicate that ion-exchange play a significant role in the retention obtained in micellar GPC [21]. This will be exposed further in Part IV thereafter.

III.2. Thin Layer Chromatography

a) Description and Advantages

The first uses of micellar phases by Armstrong were done in GPC and thin layer chromatography (TLC). This was described in Chapter 3. TLC was a useful tool for the determination of solute partition data in micellar systems. The micellar partition coefficient, P_{WM} , the solute-stationary phase interaction coefficient, P_{WS} , and the micellar binding constant, K_D , could be obtained from the solute- R_f parameters with an equation very similar to eq. 13.5 [22]. A number of solutes were separated by micellar TLC such as phenols and dyes [22], indicators, caffeine, biphenyl, naphthol and benzamide [23], PAHs and amino acids [24], vitamins [25] or fluorescein derivatives [26]. The low cost, low toxicity, peculiar selectivity and ease of

operation of micellar TLC renders the technique very useful in teaching laboratories. A number of practical demonstrations can be performed at practically no risk for the students [23].

b) Drawbacks

Surfactant adsorption on the thin layer support cannot be avoided. It is the cause of a micellar gradient concentration between the solvent front and the mobile phase reservoir. Adsorption up to saturation of the sorbent depletes the surfactant concentration in the mobile phase. A double solvent front was observed, the upper one was a dilute non micellar surfactant solution, the lower second front corresponded to the micellar front [22-25, 27]. The problem is that this phenomenon introduces potential error in the identification of the exact R_f parameters of the solutes, inducing accuracy concerns of the K coefficients [28]. This adsorption-induced micellar concentration gradient was used to separate the polar solutes in the nonmicellar region (between the two solvent fronts) from the hydrophobic solutes separated by the slow moving micellar phase [22, 27].

The typical elution times in micellar TLC are in the hour range. Such long development times are required due to the relatively high viscosity of the micellar solutions compared to organic solvents. The micellar phase viscosity was also responsible for the increase in spot size (low efficiency) [23]. Small amounts of sample should be deposited on the plates because the solubilizing power of micellar phases is lower than that of organic solvents.

The last original stationary phase used with micellar phases is the wall of capillary open tubes. The forces driving the micellar phase are not mechanical (pressure) but electrical (electric field). This is part of the world of capillary electrophoresis. It deserves special consideration found at the end of this chapter.

IV. MICELLAR PHASES AND IONS

When ionic solutes must be separated using MLC, electrostatic interactions will occur between the analytes and the ionic surfactants. Ion-pairing and/or ion-interaction can occur simultaneously with the ionic surfactant covered

stationary phase and with the mobile phase containing charged micelles. Ion-pairing complements the micelle partition mechanism described for nonionic solutes. The ion retention is the result of this multifarious mechanism.

IV.1. Inorganic Anions

a) Retention Behavior

Anions will be separated by cationic micellar phases. Kirkbright and Mullins obtained the elution order $I^- > NO_3^- > Br^- > NO_2^- > IO_3^-$ with a 0.14 M cetyltrimethylammonium chloride (CTAC) micellar mobile phase and a C18 Spherisorb column [29]. This is the usual eluotropic order obtained with strong basic anion exchangers. They showed that the ion retention decreased with the increase of both the ionic strength, μ , and the surfactant concentration. Plots of $1/k$ vs. $\mu^{1/2}$ and $1/k$ vs. [CTAC] were linear. The first linear relationship is typical of ion-exchange mechanisms, the second plot means that the anion-micelle interaction obeys the Armstrong-Nome model for molecule-micelle partition.

With a shorter chain cationic surfactant, hexadecyl trimethylammonium chloride (HTAC), and a polar stationary phase, Asahipack® GS-310H, a polyvinylalcohol gel, Okada obtained a reversed selectivity [30]. The elution order was $NO_2^- > NO_3^- > IO_3^- > I^-$. The amount of HTAC adsorbed on the polar stationary phase is in the $0.5 \mu\text{mole/m}^2$ range, one order of magnitude lower than the surfactant coverage of apolar C18 or C8 phases (see Chapter 4). The ion-exchange mechanism is less important compared to the ion-transportation by micelles. Ionic strength changes produced drastic selectivity variations [18]. At low ionic strength, small micelles interacted with the GPC gel. Micelle exclusion was the dominating mechanism. When the ionic strength was high (0.4 M NaCl or 0.2 M K_2SO_4), classic ion-exchange elution order was obtained. The high ionic strength increased both the micelle size and the surfactant adsorption. The first effect decreased the micelle-gel interaction, the second one favored the ion-exchange mechanism [18, 30]. This shows that the anion elution order depends on the mobile phase composition and the nature of the stationary phase.

b) Retention Mechanism

Taking in account three possible exchange equilibria, (i) stationary phase-bulk solution, (ii) charged micelles-bulk solution and (iii) competition for ion-exchange sites between the analyte anions and the surfactant gegenions or ions added to buffer the ionic strength, Okada derived the retention factor equation [31]:

$$\frac{\phi}{K} = \frac{[C_{aq}^-]}{K_{ieS}[C_s^-]} \left(1 + \frac{K_{ieM}[M]}{[C_{aq}^-] + K_D} \right) \quad (13.6)$$

The subscripts ieS and ieM refer to the ion-exchange equilibria at the solution-stationary phase and the solution-micelle interface, respectively. The [C] concentrations are the counter anion concentration in the aqueous phase, aq, including added salts, and the one on the stationary phase, s. K_D is the micellar counterion dissociation constant. ϕ is the column phase ratio, [M] is the micellar concentration and k is the anion retention factor. Equation 13.6 obtained from ion-exchange equilibria resembles the classical Armstrong-Nome equation. The P_{WM} partition coefficient of eq. 13.6 can be related to the K_{ieM} constant by [30]:

$$P_{WM} = \frac{K_{ieM}}{V([C_{aq}^-] + K_D)} + 1 \quad (13.7)$$

and the P_{WS} partition coefficient [31]:

$$P_{WS} = K_{ieS} \frac{[C_s^-]}{[C_{aq}^-]} \quad (13.8)$$

As soon as a moderate concentration of salt ($C \approx 0.05$ M) is added to the micellar phase, the P_{WM} and P_{WS} values can be regarded as constants when the micellar concentration changes. Table 13.32 lists the K_{ieM} , K_{ieS} and P_{WM} values obtained for some ions [30, 31]. The K values depend on the micellar concentration but not on the added salt concentration. The P_{WM} constant is independent of the micellar concentration but decreases if the ionic strength increases.

Table 13.2 Partition Versus Ion-Exchange to Model Anion Micellar Interaction.

Anion	Added NaCl	K_{ieS} M^{-1}	K_{ieM} M^{-1}	P_{WM}
BrO_3^-	0.1M	1.77	1.37	35
Br^-	0.1M	4.32	2.77	60
I^-	0.1M	30.1	12.1	615
	0.2M			341
	0.3M			226
NO_2^-	0.1M	2.16	1.74	38
	0.2M			24
	0.3M			16
NO_3^-	0.1M	6.17	3.53	100
	0.2M			57
	0.3M			43

Micellar phase: CTAC 0.08M, Data from [30].

IV.2. Cationic Metal Species

a) Retention Behavior

It is difficult to separate cations of the same charge by LC. Ligands are added to the mobile phase to form metallic complexes. This enhances the ion-exchange selectivity in MLC. Since complexation can produce charged or noncharged species that interact with all micelles, the interpretation of the

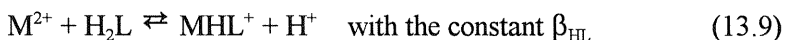
retention data in MLC of cations is difficult [30]. Complexes can be involved in ion-exchange equilibria and micellar partitioning simultaneously. Anionic micellar phases will interact with cationic species but cationic micellar phases can be used as well to separate neutral or negatively charged metal complexes.

Sodium diethyldithiocarbamate (DDTC) was used to separate transition metal cation with a CTAB micellar phase and a C18 column [32]. The limits of detection obtained with atomic absorption spectroscopy were in the tens of picograms injected. Since a high concentration of *l*-propanol (45% v/v) was added to the 0.03 M CTAB mobile phase, the presence of micelles may be discussed. Simple ion-pairing between CTAB and the DDTC metal species may explain the observed selectivity. Tartaric acid was also used as a ligand for transition metal cations with a SDS micellar mobile phase and a C18 column [33].

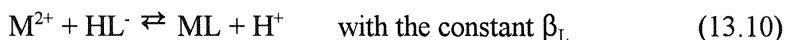
Okada showed that it was possible to simultaneously separate non-ionic compounds and cations in a complexed form [34, 35]. The separation of phenols was optimized on a C18 column with a SDS micellar mobile phase. Next, α -hydroxyisobutyric acid was added to the SDS micellar phase as a ligand for rare earth cations. This mobile phase was able to separate, at the same time, a mixture of 10 phenols and 15 cations as shown by Figure 13.6.

b) Retention Mechanism

If the cations are separated directly with an anionic micellar mobile phase, the equations derived for the MLC anion separation (eqs. 13.6, 13.7 and 13.8) can be used. Most often a complexing agent, a ligand H_2L , is added to the micellar solution forming the following equilibria with the cation, M^{2+} :



and



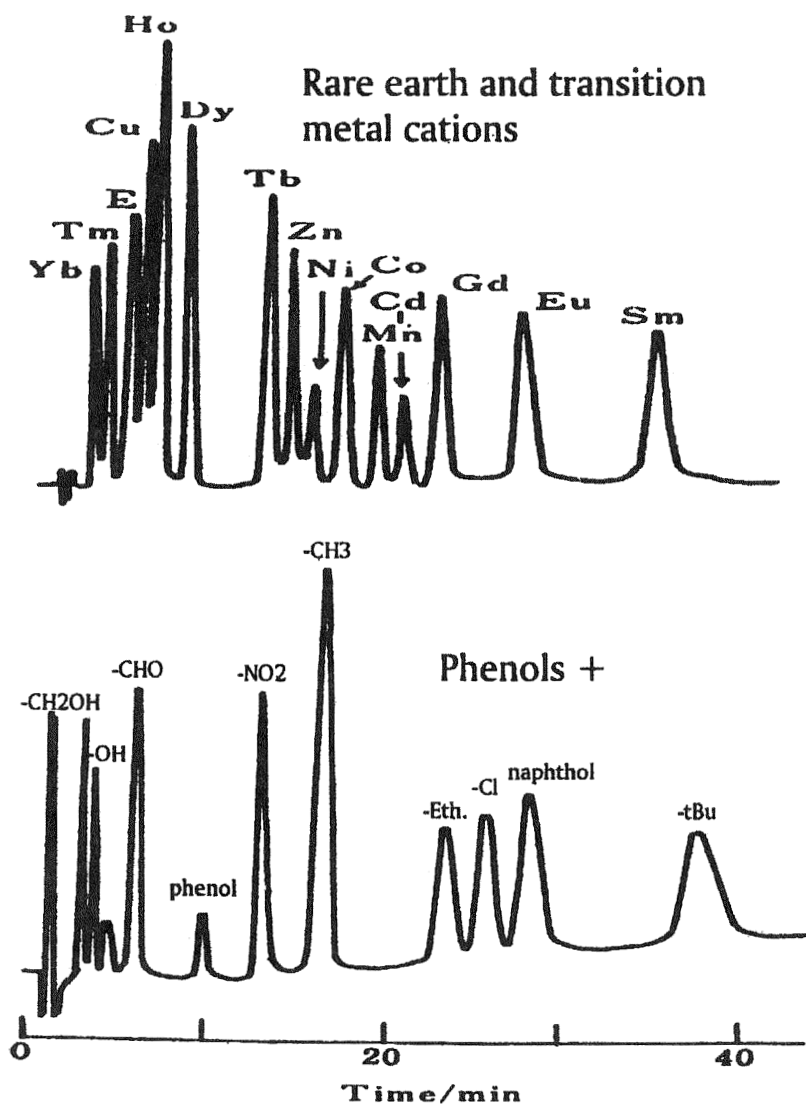
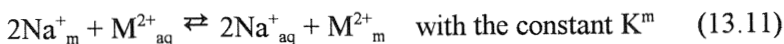


Figure 13.6 Simultaneous separation of para substituted phenols (Bottom: detection UV@280 nm) and cations (Top: postcolumn derivatization + visible 540 nm). Column: 15 cm $\times$ 4.6 mm i.d., 5 μ m Inertsil ODS-2, mobile phase: 0.1 M SDS + 0.08 M hydroxyisobutyric acid, pH 4.05, 1 mL/min. Adapted from [35].

Sodium ions buffering the ionic strength and/or added as counterions of pH buffer salts compete with the analyte cations. Ion-exchange with micelles:



and a similar ion exchange with the stationary phase (constant K^s) can take place. Considering these exchanges, Okada derived the equation for the apparent cation micellar partition coefficient [30, 33]:

$$P_{WM}^{app} = \frac{K^m}{1 + [L^{2-}](\beta_{HL} \frac{[H^+]}{K_2} + \beta_L)} \times \frac{[Na^+]_m^2}{[Na^+]_{aq}^2} \quad (13.12)$$

and the corresponding equation for the apparent P_{ws} coefficient changing the sub- or superscripts m by s [33]. In these equations, K_2 is the second dissociation constant of the ligand molecule, H_2L (tartaric acid, for example [30]). Experimental measurements showed some discrepancy with the predicted results. This was due to the shift of the dissociation equilibria of the H_2L molecule in the presence of micelles. Once the micellar ΔpK_a shift was taken in account, the cation retention with micellar complexing mobile phases was correctly predicted [30, 33-35].

IV.3. Conclusion

Simple ions can be separated by micellar mobile phases. Although most of the ion retention can be interpreted on the basis of ion-exchange models, the physicochemical meaning of such models is a little doubtful [30]. Separations of ions with probably nonmicellar phases containing very high concentration of organic solvents, the omission of solvation effects, micelle changes in size, shape or ionization state or local heterogeneities are not explained by

the present models. However, these models have proved their usefulness for the prediction and optimization of ion MLC separations. Utilization of new original surfactants, functionalized micelles or micellar catalysis will give rise to further possibilities in the MLC of inorganic ions still modeled with simple ion-exchange interactions [30].

V. MICELLAR SEPARATIONS WITHOUT A CLASSIC COLUMN

The column is the core of the LC separation technique. To expand the field of MLC, a rapid description of two separation techniques that do not use a column, but can use a micellar phase is presented. The two techniques are field flow fractionation (FFF) and capillary electrophoresis (CE).

V.1. Micellar Field Flow Fractionation

a) Principle of FFF

FFF was introduced by Giddings in 1966 to separate “objects” such as proteins, particles, latexes, cells, polymers, powders and other macromolecules. FFF uses an external field applied on a long ribbonlike channel. The injected “objects” are driven toward the lower wall of the channel. Because of the parabolic flow profile in the channel, the objects forced against the wall travel more slowly and are retained relative to the solvent or objects that interact less or do not interact with the field. The range in molecular weight is 10^4 to 10^{16} [36]. Armstrong and Berthod thought that micelles and/or microemulsion droplets were “objects” falling in the FFF molecular weight range. So, a micelle or a microemulsion droplet could be retained in a FFF channel. Since small molecules can partition with the micelle or microemulsion droplet, they could also be retained in the FFF channel by secondary chemical equilibrium as illustrated by Figure 13.7. This would extend the applicability of the FFF technique to small molecules. These two authors studied the theoretical feasibility of the use of a micellar mobile phase in a FFF channel [37].

b) Separation of Small Molecules by Micellar FFF

The theoretical study showed that micelles or microemulsions droplets should be retained in an FFF channel. Injected solutes should partition between the aqueous nonretained and the organic retained pseudo-phases. The solutes are more or less retained according to their partition coefficient, P . The solute retention time is:

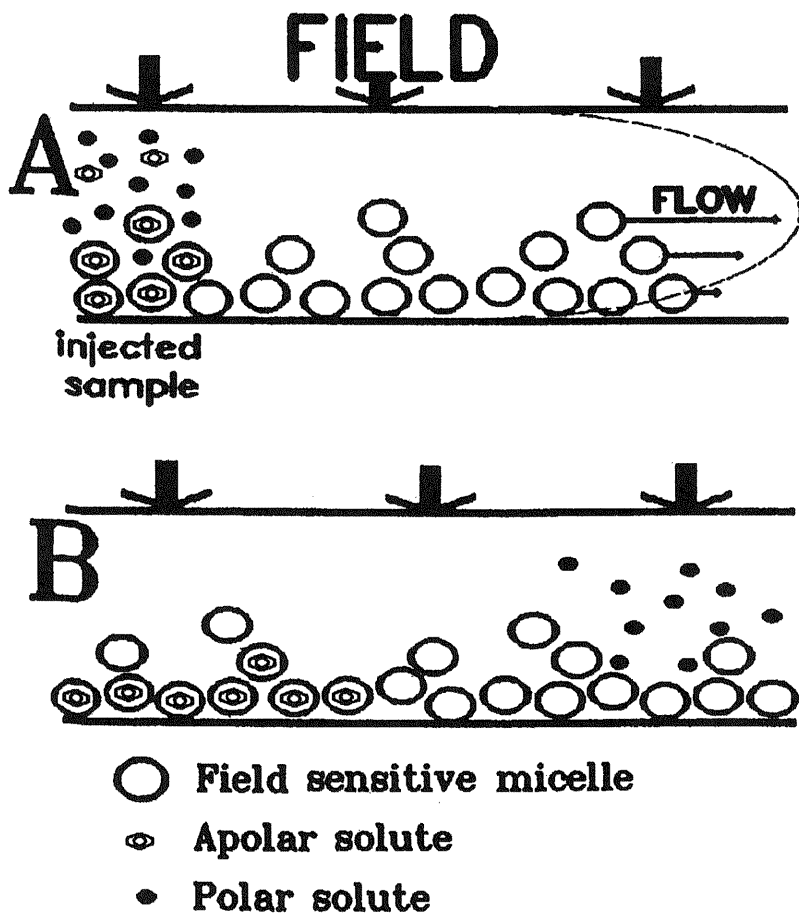


Figure 13.7 Principle of micellar FFF. Field sensitive micelles move more slowly than the carrier. A: sample injection; B: the polar solute (dark circles) moves with the carrier, the apolar solute (hexagons) moves with the micelles. Reprinted from Ref. 38 with permission of the American Chemical Society.

$$t_r = t_o \frac{W}{6L} \times \frac{1}{\coth\left(\frac{W}{2L}\right) - \frac{2L}{W}} \quad (13.13)$$

in which t_o is the dead time, W is the channel width and L is the average distance of the solute from the wall. This distance depends on the solute partition coefficient, but also on the field strength and on the micellar concentration in the carrier [37].

Figure 13.8 shows the first separation of small molecules by FFF. Ascorbic acid was separated from toluene through a secondary chemical equilibrium with field-retained microemulsion droplets. Once again, the exchange between the aqueous phase and the swollen micelles is low, *i.e.*, the efficiency is low and broad peaks are obtained (Figure 13.8). There are so many powerful techniques for small molecule separation that micellar FFF was not used for this purpose. Its interest could be in the physicochemical study of the micellar or microemulsion structure. For example, in the case of the Figure 13.8 experiment, the separation allowed the estimation of the average mass of the mobile phase microemulsion droplets (1.4×10^{-16} g) and consequently, its radius (35 nm) [38]. These values can be obtained by heavy methods such as small angle neutron scattering or high resolution NMR [38]. Micellar FFF can be an easy alternative in such studies.

V.2. *Capillary Electrophoresis with Micellar Phases*

The capillary electrophoresis (CE) technique can be used with micellar phases. The first use of such phases with CE was presented in 1984 by Terabe, who called the technique: micellar electrokinetic chromatography (MEKC) [39]. Its success was tremendous because it opened the use of CE to noncharged molecules and species. CE became an essential separation tool especially in the field of biology. Today ten applications are published in MEKC for only one in MLC. Numerous books and review articles describe the CE technique including the use of micellar phases [40-43]. A simplified survey is presented here.

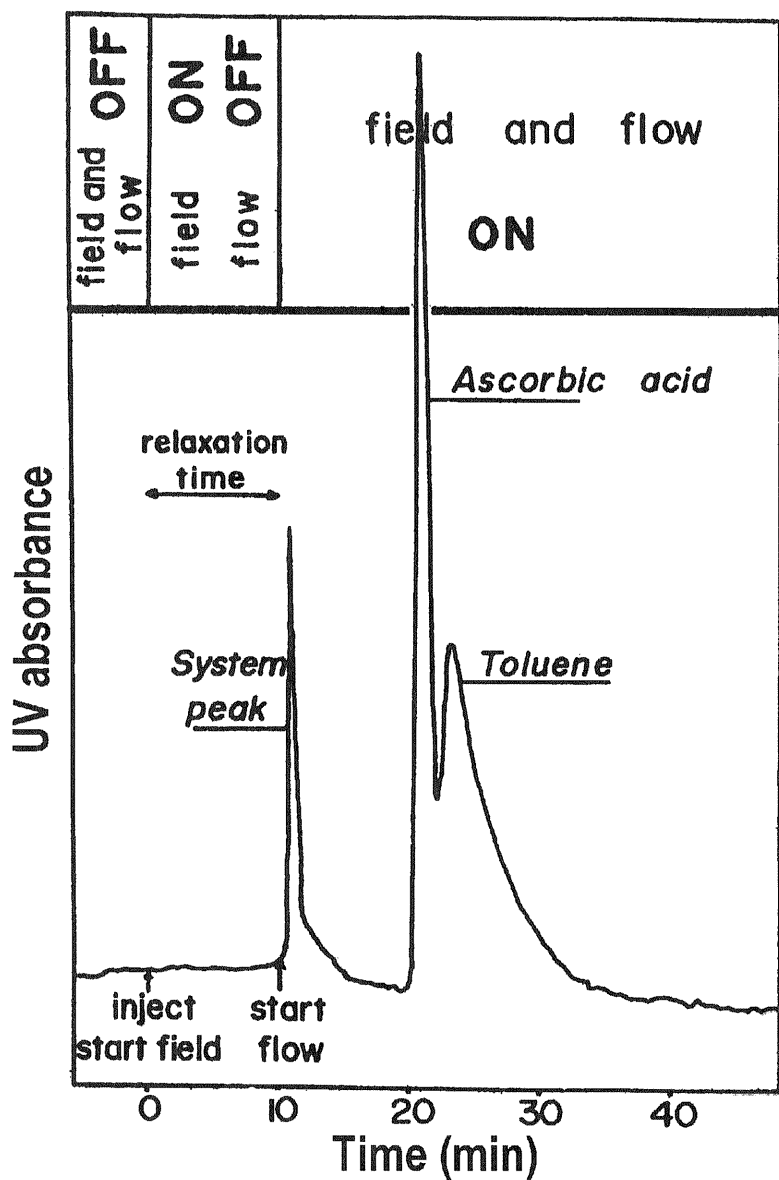


Figure 13.8 The first micellar FFF separation. Rotation speed 1400 rpm, field 337 G, flow rate 0.22 mL/min. The system peak is the detector response with flow changes. Reprinted from Ref. 38 with permission of the American Chemical Society.

a) Principle

CE exists in a strong electric field (4 to 70 kV/m) applied in an open-tubular silica capillary (i.d. 20 to 100 μm , length 20 to 100 cm) filled by an electrolyte. Two small beakers receive the capillary ends and the two platinum electrodes of a high voltage unit that generates the electric field.

Electroosmotic Flow. Due to surface charges on the capillary wall, an electroosmotic flow is generated by the electric field. This flow depends on the strength of the electric field, the temperature, the pH, the ionic strength, the solvent (viscosity and dielectric constant) and the nature of the charges on the capillary wall. Without surfactant and with anionic surfactants in the mobile phase, the electroosmotic flow is oriented toward the negative electrode (cathode). The electroosmotic flow does not have the parabolic profile of any laminar flow in an open tube, rather its profile is flat. A narrow band injected at the beginning of the capillary travels with the electroosmotic flow without broadening due to the parabolic flow profile.

Electrophoretic Mobility. Any charged species moves in an electric field toward the electrode of the opposite charge. The electrophoretic mobility of the charge is related to its speed in the electric field. The electrophoretic mobility depends on the pH that can change the ionization state of the species, and on the ionic strength and viscosity of the electrolyte medium. The electrophoretic mobility of an ion can be in the same direction or in an opposite direction as the electroosmotic flow depending on its charge. Neutral solutes are not sensitive to the electric field, they move with the electroosmotic flow. Micelles of ionic surfactant are charged so they have an electrophoretic mobility. Neutral solutes can partition with the micelles. The solutes will be separated according to their micellar affinity. Figure 13.9 illustrates the MEKC principle.

b) Chromatographic Parameters

Retention. Micelles have a retention time, t_{mc} , depending on their charge and size. A neutral solute that does not interact with the micelles, moves with the electroosmotic flow. Its retention time is t_{eo} . All the other neutral solutes have a retention time, t_{R} , between these two values that delineate the time window, $t_{\text{mc}} - t_{\text{eo}}$, of the technique. The retention factor, k , is expressed by [39]:

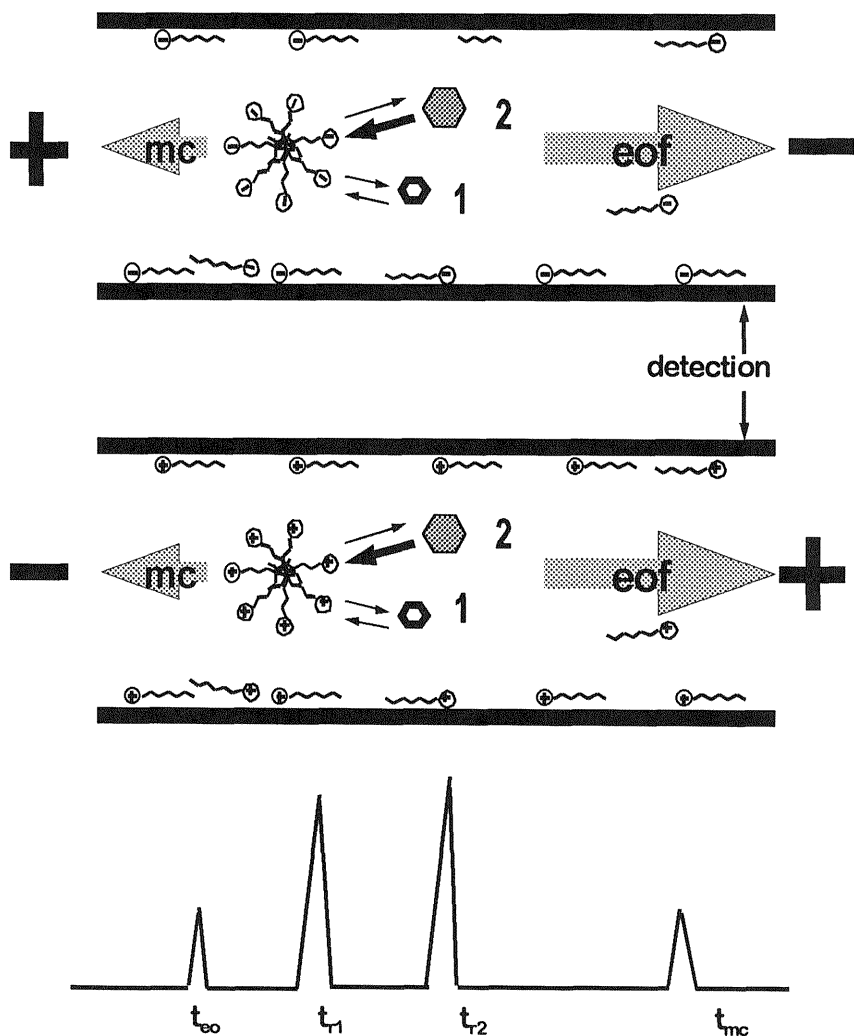


Figure 13.9 Principle of MEKC. Top: anionic micelles, the electroosmotic flow has the anode (+) to cathode (-) direction. The anionic micelles are attracted by the cathode (+). Bottom: cationic micelles. Everything is reversed. Solute 1 (open bold hexagons) has a lower affinity constant for the micellar phase than Solute 2 (filled hexagons). eof = electroosmotic flow, mc = electrophoretic micelle motion. Adapted from [46].

$$k = \frac{t_R - t_{eo}}{t_{eo}(1 - (t_R/t_{mc}))} \quad (13.14)$$

k is proportional to the solute micelle affinity coefficient, P_{WM} , and to the surfactant concentration, C_s [42]:

$$k = P_{WM} v (C_s - cmc) = P_{WM} v [M] \quad (13.15)$$

In MEKC, the retention factor is not proportional to the retention time. When the solute affinity for the micelle is very high (large K_{MW}), the retention factor is very high, the retention time, t_R , is not high but close to the micelle retention time, t_{mc} .

Efficiency. Efficiencies in the hundred of thousands plate/m range are common in MEKC. This is due to the square nature of the electrophoretic flow profile. The mobile phase flowing does not induce any band broadening as it does in all other chromatographic techniques. Longitudinal diffusion is the only physicochemical cause of band broadening. In this situation, the low diffusion coefficient of the bulky micelles that was a problem in MLC (see Chapter 6) turns into a big advantage in MEKC. A solute located inside a micelle has the low diffusion coefficient of the micelle itself while it is retained. Consequently the observed peak of this solute is very sharp. However, if the plate numbers obtained with MEKC (~100,000 plate/m range) is impressive when compared to those obtained with MLC (~10,000 plate/m range), it is not as high as what can be achieved with classic CE that can approach the million plate/m. The resistance to mass transfer that is introduced by solute partitioning between the bulk buffer and the micelles was greatly responsible for the low MLC efficiency. It is again responsible for the efficiency difference between MEKC and classic CE separations [44, 45].

Resolution. The resolution equation between Solutes 1 and 2 is [40, 42]:

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_2}{1 + k_2} \right) \left(\frac{1 - t_{eo}/t_{mc}}{1 + k_1(t_{eo}/t_{mc})} \right) \quad (13.16)$$

In eq. 13.6, the three first terms are the classic Snyder resolution equation for HPLC. A high efficiency and a high selectivity factor are favorable to obtain a useful resolution. There is, however, an optimum range of k due to the limited elution window, around $1 < k < 5$, within which maximum resolution can be achieved. Outside this optimum, *i.e.*, for poor or strong retained solutes, resolution decreases rapidly [46]. The last term of eq. 13.16 is specific to MEKC. It takes in account the t_{eo}/t_{mc} ratio that corresponds to the time windows of the technique. A large time window means $t_{mc} \gg t_{eo}$ so the ratio t_{eo}/t_{mc} is very small. This is a favorable condition for a high resolution. Usually anionic surfactant micelles produce larger time windows than cationic micelles. The separation capabilities of MEKC is higher with anionic micelles than those with cationic micelles [47].

c) Practical Operations

A primary reason for the exploding interest in MEKC, both in the academic and industrial area, is its combination of high efficiency, versatility, speed, ease of use and adjustable selectivity.

Optimization. Acting on the instrumental parameters is the first way to optimize a separation. The applied voltage, the capillary dimensions and the injection mode should be optimized. The temperature should not vary and stay low. The current should be kept low (in the tens μA range) to avoid heating by the Joule effect. This is done by adjusting the electrolyte concentration and composition. Analysis reproducibility can be enhanced by careful cleaning of the capillary between runs.

MEKC can also be optimized by controlling the migration behavior of the solute. This is done through manipulation of the P_{WM} solute binding constants and change of the elution windows. The type of surfactant, its

concentration, and the addition of modifiers such as organic solvents, cyclodextrins, urea or even another surfactant (mixed micelles) to the mobile phase are some ways to control the solute migration behavior [48]. Introduction of secondary chemical equilibria in the mobile phase, the primary equilibrium being partitioning with the micelle, can also greatly change the selectivity in MEKC. pH changes, ion pairing or complexation equilibria are examples of such possible secondary equilibria allowing to enhance a separation [41, 42, 46, 48]. Most of these changes will affect some physicochemical parameters of the electrolyte, viscosity, conductivity or dielectric constant that will modify the current (Joule heating) or the electroosmotic flow direction and/or intensity. The instrumental parameters should consequently be adjusted.

Applications. Figure 13.10 shows the electropherograms of a complex mixture of pharmaceutical principle with analgesic, antipyretic and antitussive properties obtained with different micellar phases. It shows the effect of different micellar phases on the selectivity and resolution of the analysis. Beside pharmaceutical separation, MEKC has been used in thousands of different separations in practically all chemistry related fields such as the biological field, amino acids, peptides and nucleic acid constituents; the biomedical and personal care field, pharmaceuticals, cosmetics, metabolites, vitamins and clinical applications; the chemical engineering field, organic acids and bases, hydrophobic and structural isomers, inorganic and organometallic compounds; the agrochemical and food field, food content, sugars, glucosides, fatty acids and lipids; the environmental field, priority pollutants, PAHs, PCBs, water analysis and research with separation of chiral molecules [39-50].

In conclusion, both the great resolving power and the ease of use of MEKC explain the great interest that this technique receives. The limited elution windows is a disadvantage, however, the problem is greatly improved through the use of mixed micelles, polymeric phases and organic modifier additions. Most recent bench top mass spectrometers with electrospray introduction are able to work directly with MEKC without quick contamination of the ion-source. The MEKC-MS coupling will further broaden the scope of the technique.

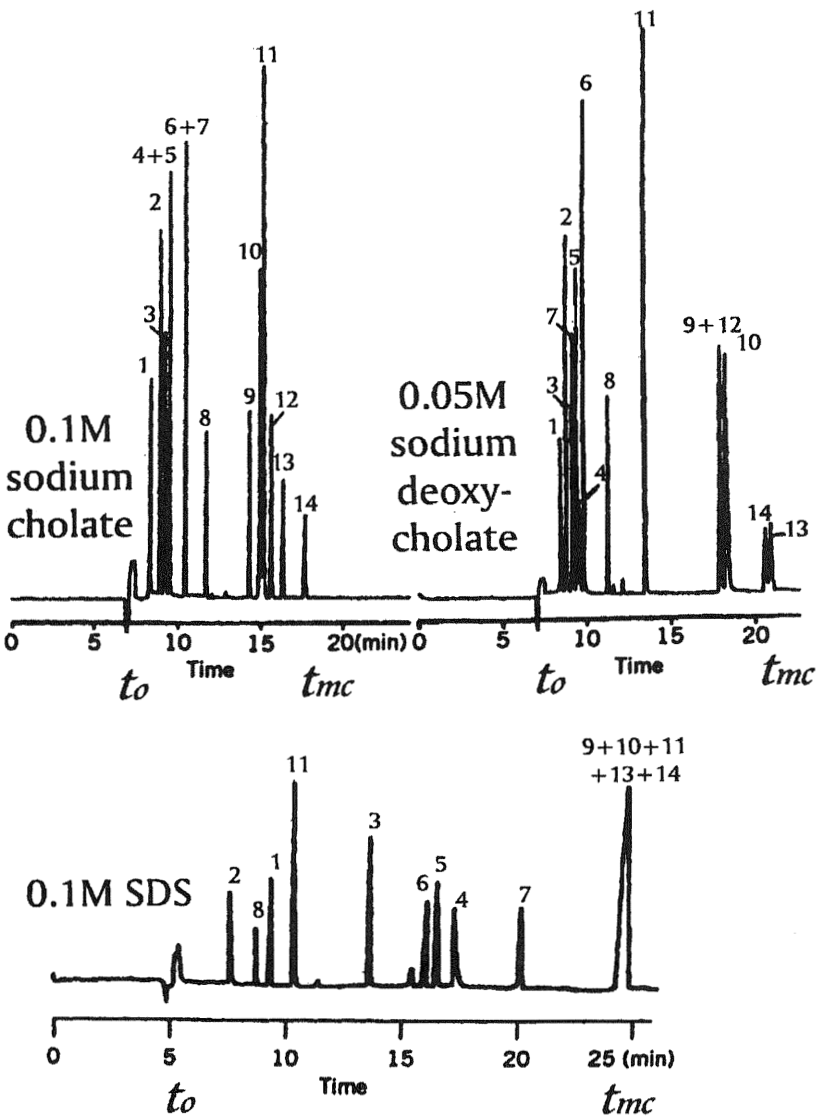


Figure 13.10 Effect of the surfactant nature on the electropherograms of a complex antitussive preparation containing 14 solutes (analgesic, antitussive, antipyretic principles, conservatives and excipients). Buffer: 0.02 M phosphate-borate, pH 9. Capillary 50 μ m, 65 cm fused silica. Detection UV 210 nm. Adapted from [47].

VI. CONCLUSION

It is perhaps *comme il faut* to finish this book with MEKC, the analytical technique that is most successful with micellar phases. At the moment, the latest separation technique still under development is electrochromatography (EC). EC is a microchromatography technique with filled capillary columns in which the pump is replaced by an electric field. The electroosmotic flow is solely responsible for mobile phase motion. The flat flow profile passes the million plate/m in efficiency. Neutral solutes are retained through interaction with the stationary phase. There is no theoretical objection to the use of micellar mobile phases with EC. Unfortunately, it can be predicted that surfactant will adsorb on the stationary phase and that the solute mass transfer between the bulk and micellar phase will be slow. The efficiency obtained with micellar EC will be likely lower than that obtained with EC and hydro-organic mobile phases. However, the adsorption of ionic surfactants will completely change the stationary phase surface potential, consequently it will change the electroosmotic flow and may produce original and interesting results.

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Appendix I

MICHROM Software

The enclosed CD-ROM contains everything needed to run MICHROM. This software is able to take the results obtained with a set of compounds and several compositions of hydro-alcoholic micellar phases, and calculate the affinity constants to predict the results for compositions of mobile phase (surfactant concentration, modifier concentration and pH).

MICHROM includes the first module, MICHROM1, used to enter and process the data for the user selected model and to generate a series that can be used by MICHROM2. The second module can produce contour plots, 3D surfaces, chromatograms for a given composition and even dynamic simulations.

The required first step is to read the User's Manual found on the CD-ROM. Acrobat Reader®, from Adobe Systems Incorporated, can be installed from the CD. It should be used to visualize and/or print the User's Manual PDF file. It is recommended to print the 40 pages of the user's manual titled "Getting Started with MICHROM." The figures of the MICHROM user's manual are in color. They can be seen on a color screen using Acrobat Reader® and can be printed as such with a color printer.

It is possible to try MICHROM directly from the CD-ROM using one of the .CRS files loaded on the CD-ROM (fases81.CRS, mic1.CRS or sulfa11.CRS). In order to process a personal set of data, it is necessary to install MICHROM1 and 2 onto your hard disk. The installation is straightforward by just copying the MICHROM files into a dedicated directory.

Appendix II

Critical Micelle Concentrations of Selected Surfactants

The chromatographer is always wondering where he or she can get the cmc of a particular surfactant. Since MLC makes exclusive use of surfactant solution above the critical concentration, we thought the reader would appreciate using our collection of cmc values. When the physical parameters needed in MLC were known, they were included. This table was prepared by and is intended for chromatographers. Physico-chemists would complain that the methods used to obtain the cmcs were not listed. Furthermore, most of the values came from previous compilations, especially the 1993 compilation of Van Os, Haak and Rupert (1) and the NSF (now NIST) compilation of 1971 by Mukerjee and Mysels (2). These references contain the original references for the methods used.

The table is sorted first by global surfactant formula, then by increasing temperature and finally by additive. When several different values for the cmc of a particular surfactant were found, further references were examined (see Additional Reference list) and the most often cited value was selected. This would be listed in Ref. 2 as a “questionable criterion.”

The 13 columns give successively: 1) the global surfactant formula, 2) its molecular weight, 3) its charge with **N** for nonionic, **M** for mixed (both the anion and the cation have surfactant properties), **Z** for zwitterionic, and of course, + and - for cationic and anionic surfactants,

4) the temperature, 5) the **cmc** in mole/L, 6) and 7) the medium with nature and concentration, respectively, 8) the molar volume in L/mole, 9) the micelle aggregation number, 10) the Krafft temperature for ionic surfactant or the cloud point of a 10 g/L solution in °C, 11) the counter ion binding, *e.g.*, 0.65 for SDS whose micelle has 62 molecules means that 65% of the 62 molecules have a sodium ion associated, the micelle bears about 22 negative charges, 12) the reference and 13) the surfactant chemical name.

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C6H13NaO3S	188	-	20	0.44					<0		1	sodium hexylsulfonate
C8F15KO2	352	-	25	0.029							2	potassium perfluoroheptanoate
C8H15KO2	182	-	25	0.4 0.345	KOH	0.033 M				0.58	2 4	potassium octanoate
C8H15NaO2	166	-	20	0.28						0.58	2	sodium octanoate
C8H17NaO3S	216	-	25	0.155				25			3	sodium octylsulfonate
C8H17NaO4S	232	-	25 40 60	0.13 0.14 0.146				24		0.46 0.46	2 3 2	sodium octylsulfate
C8H20ClN	166	+	25	0.12							2	octylammonium chloride
C9H19NaO4S	246	-	20 21	0.065 0.0406	NaCl	0.1 M		31			1 4	sodium nonylsulfate
C10H19KO2	210	-	25	0.1						0.58	2	potassium decanoate
C10H19NaO2	194	-	20 25	0.07 0.094						0.58	2 4	sodium decanoate
C10H21NaO3S	244	-	25 40	0.043 0.041				40	22		4 3	sodium decylsulfonate
C10H21NaO4S	260	-	25 60	0.032 0.035				38		0.46 0.46	2 2	sodium decylsulfate
C10H21NaO4S	260	-	25	0.046							1	sodium decyl 2-sulfate
C10H22O2	174	N	25	0.0049							1	ethylene glycol mono octyl ether
C10H24ClN	193	+	25	0.035							2	decylammonium chloride
C10H4F19NO2	532	-	25	0.038							3	ammonium perfluorononanoate

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C11H18BrN	244	+	25	0.0048							1	hexylpyridinium bromide
C11H23NaO3S	258	-	25	0.0135							1	sodium undecylsulfonate
C11H23NaO4S	274	-	20	0.016	NaCl	0.1 M		45	7	7	1	sodium undecylsulfate
			21	0.016							4	sodium undecyl sulfate
			21	0.0054							4	sodium undecyl sulfate
C11H25NO	187	N	25	0.0054							4	nonyl dimethylamine oxide
C11H26BrN	252	+	25	0.21							2	octyltrimethylammonium bromide
			60	0.28							2	
C11H26ClN	208	+	25	0.039	NaCl	0.1M					2	octyltrimethylammonium chloride
C12H17NaO3S	264	-	75	0.037				38			1	sodium hexyl benzene sulfonate
C12H23KO2	238	-	25	0.027	KCl	0.05 M				0.58	2	potassium dodecanoate
			25	0.013							2	
			25	0.009							2	
			25	0.006							2	
C12H23NaO2	222	-	20	0.018						0.58	2	sodium dodecanoate
C12H25KO4S	304	-	25	0.0078					34		3	potassium dodecylsulfate
C12H25LiO4S	272	-	25	0.0089							3	lithium dodecylsulfate
C12H25NO2	215	Z	27	0.25							3	octyl betaine
			60	0.095							3	
C12H25NaO3S	272	-	25	0.0088			0.229	54	31		4	sodium dodecylsulfonate
			40	0.0097							4	
C12H25NaO4S	288	-	10	0.0087					9		4	sodium dodecylsulfate, SDS
			15	0.0084					9		4	
			25	0.0082			0.246	62	10	0.65	3	

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C12H25NaO4S	288	-	25	0.0056	NaBr	0.01M	0.246	92		0.95	4	sodium dodecylsulfate, SDS
			25	0.0056	NaI	0.01M					4	
			25	0.0056	NaF	0.01M					4	
			25	0.0014	NaCl	0.1M					5	
			25	0.009	dioxane	10% v/v					4	
			25	0.03	dioxane	25% v/v	0.246	60		0.6	4	sodium dodecylsulfate and alcohols
			25	0.008	MeOH	5% v/v					5	
			25	0.008	MeOH	12% v/v					13	
			25	0.008	MeOH	24% v/v					13	
			25	-	MeOH	40% v/v					13	
			25	0.006	EtOH	10% v/v					4	
			25	0.01	EtOH	20% v/v					13	
			25	-	EtOH	30% v/v					13	
			25	0.0043	2-PrOH	6% v/v				0.6	4	
			25	0.0038	1-PrOH	6% v/v				0.6	4	
			25	-	1-PrOH	22% v/v				13		
			25	0.0072	BuOH	0.5% v/v				0.5	13	
			25	0.0048	BuOH	1.5% v/v				0.45	13	
			25	0.004	C5OH	1% v/v				0.45	13	
			25	0.0044	C6OH	0.3% v/v				0.45	13	
			25	0.0048	C7OH	0.1% v/v				0.55	13	
			25	0.0072	NaCl	0.001 M					1	sodium dodecylsulfate and NaCl
			25	0.0061	NaCl	0.005 M					2	
			25	0.0049	NaCl	0.01 M					1	
			25	0.0031	NaCl	0.03 M					1	
			25	0.0016	NaCl	0.1 M					1	

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C12H25NaO4S	288		25	0.0007	NaCl	0.3 M		105			1	sodium dodecylsulfate, SDS
			45	0.009							4	
			60	0.0098							2	
C12H26O4	234	N	20	0.01					48		1	polyoxyethylene3 hexyl ether
C12H28ClN	222	+	25	0.015	NaCl	0.05 M					4	dodecylammonium chloride
			25	0.0067							4	
			25	0.024		MeOH					4	
			25	0.0092		2-PrOH					4	
			25	0.0074		tBuOH					4	
C13H19NaO3S	278	-	25	0.0228					9		1	sodium heptyl benzene sulfonate
C13H22BrN	272	+	25	0.0063				24			1	octylpyridinium bromide
C13H27NaO3S	286	-	25	0.0035								sodium tridecyl sulfonate
C13H30BrN	280	+	25	0.065	NaCl	0.1 M	0.281	39			4	decyltrimethylammonium bromide
			25	0.05							2	
			25	0.04		0.3 M					2	
			55	0.058		1 M					1	
			60	0.074							2	
C13H30ClN	236	+	25	0.061	NaCl	0.1 M					3	decyltrimethylammonium chloride
			25	0.0085							2	
C14H21NaO3S	292	-	25	0.011				50	19		1	sodium octylbenzene sulfonate
			35	0.0158							1	
			35	0.0147							4	
			50	0.0135							1	
			55	0.23							2	

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C14H25NaO7S	360	-	25	0.053							4	sodium dipentyl sulfosuccinate
C14H27KO2	266	-	20	0.0064							2	potassium tetradecanoate
			20	0.004	heptanol	0.1% v/v				0.58	2	
			20	0.0039	hexanol	0.4% v/v				0.58	2	
			20	0.003	pentanol	1.5% v/v				0.58	2	
			20	0.0025	butanol	4% v/v				0.58	2	
			20	0.003	propanol	9% v/v				0.58	2	
			20	0.004	ethanol	17% v/v				0.58	2	
			25	0.007							1	
			45	0.0074							2	
			65	0.0086							2	
C14H27NaO2	250	-	20	0.0045						0.58	2	sodium tetradecanoate
C14H29NO2	243	Z	27	0.013				34			3	decyl betaine
C14H29NaO3S	300	-	40	0.0025			0.297	80			3	sodium tetracylsulfonate
C14H29NaO4S	316	-	25	0.002				138	21	0.46	2	sodium tetradecylsulfate
			60	0.0027						0.46	2	
C14H29NaO4S	316	-	25	0.0051							4	sodium tetradecyl 4-sulfate
C14H29NaO4S	316	-	25	0.0033							4	sodium tetradecyl 2-sulfate
C14H29NaO4S	316	-	40	0.0067							1	sodium tetradecyl 5-sulfate
C14H29NaO4S	316	-	40	0.0097							1	sodium tetradecyl 7-sulfate
C14H29NaO4S	316	-	40	0.0043							1	sodium tetradecyl 3-sulfate
C14H30O4	262	N	25	0.0075				95	10		4	polyoxyethylene3 octyl ether
C14H30O5	278	N	20	0.09					60		1	polyoxyethylene4 hexyl ether
C14H30O6	292	N	25	0.025							4	octyl beta D-glucoside
C14H31ClN	250	+	25	0.003							2	tetradecylammonium chloride

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C15H23NaO3S	306	-	25 60	0.0103 0.0128				42	<1		1 1	sodium 3-nonyl benzenesulfonate
C15H23NaO3S	306	-	75	0.0065				63			1	sodium nonyl benzenesulfonate
C15H26BrN	300	+	30	0.048							1	decylpyridinium bromide
C15H26ClN	256	+	25	0.087							1	decylpyridinium chloride
C15H31NaO3S	314	-	45	0.0015							1	sodium pentadecyl sulfonate
C15H31NaO4S	330	-	40	0.0017							1	sodium pentadecyl 2-sulfate
C15H31NaO4S	330	-	40	0.0066							1	sodium pentadecyl 8-sulfate
C15H31NaO4S	330	-	40	0.0034							1	sodium pentadecyl 5-sulfate
C15H31NaO4S	330	-	40	0.0022							1	sodium pentadecyl 3-sulfate
C15H34BrN	308	+	20	0.0065	BuOH	5% v/v	0.308	54			1	dodecyltrimethylammonium bromide
			25	0.013							2	
			25	0.013	EtOH	10%v/v					1	
			25	0.0005	BuOH	10% v/v					1	
			25	0.016	EtOH	25%v/v					1	
			25	0.002	NaBr	0.5 M					4	
			25	0.045	urea	6 M					4	
			55	0.014	NaBr	1 M					1	
			60	0.19							2	
C15H34ClN	264	+	25	0.02				47			3	dodecyltrimethylammonium chloride
			25	0.0019	NaCl	0.1 M		56			2	
C16H25NaO3S	320	-	20	0.0044				63	16		1	sodium decyl 2-benzene sulfonate
C16H25NaO3S	320	-	20	0.0081				17	<1		1	sodium decyl 5-benzene sulfonate
C16H25NaO3S	320	-	20	0.006				53	4		1	sodium decyl 3-benzene sulfonate
C16H25NaO3S	320	-	50	0.004				72	39		1	sodium decylbenzene sulfonate

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
			70	0.051				74			1	
C16H26O2	250	N	25	0.0001							1	p-octylphenol monoxyethylene glycol ether
C16H28BrN	314	+	30	0.0195				42			1	undecylpyridinium bromide
C16H29NaO7S	389	-	25	0.0124							4	sodium dihexyl sulfosuccinate
C16H31KO2	294	-	25	0.0019						0.58	2	potassium hexadecanoate
C16H31NaO2	278	-	20	0.0011						0.58	2	sodium hexadecanoate
C16H33NO2	271	Z	27	0.0013				73			3	dodecyl betaine
C16H33NaO3S	328	-	40	0.00074			0.34				1	sodium hexadecylsulfonate
C16H33NaO4S	344	-	40	0.0024							3	sodium decylpentylsulfate
C16H33NaO4S	344	-	40	0.0017							1	sodium hexadecyl 4-sulfate
C16H33NaO4S	344	-	40	0.0023							1	sodium hexadecyl 6-sulfate
C16H33NaO4S	344	-	40	0.0009							3	sodium 2-ethyltetradecylsulfate
C16H33NaO4S	344	-	40	0.0042							1	sodium hexadecyl 8-sulfate
C16H33NaO4S	344	-	40	0.0008							3	sodium 2-methylpentadecylsulfate
C16H33NaO4S	344	-	40	0.0011							3	sodium 2-propyltridecylsulfate
C16H33NaO4S	344	-	40	0.0017							3	sodium dodecylpropylsulfate
C16H33NaO4S	344	-	40	0.00058					31	0.46	2	sodium hexadecylsulfate
			60	0.00074						0.46	2	
C16H34MgO6S2	411	-	23	0.11							4	magnesium octylsulfonate
C16H34O4	290	N	25	0.0008			0.229		<0		6	polyoxyethylene3 decyl ether
C16H34O5	306	N	25	0.0035	SDS	0.0017M	0.306		34		1	polyoxyethylene4 octyl ether
C16H34O6	322	N	20	0.09					75		1	polyoxyethylene5 hexyl ether
C16H34O6	322	N	25	0.0022							4	decyl beta D-glucoside
C16H34O7	338	N	20	0.8							3	polyoxyethylene6 butyl ether
			40	0.71							3	

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C16H36CIN	278	+	25	0.00085							2	hexadecylammonium chloride
C16H37NO4S	339	-	25	0.0055							4	tetramethylammonium dodecylsulfate
C17H27NaO3S	334	-	60	0.002				87	44		1	sodium undecylbenzenesulfonate
C17H30BrN	328	+	25	0.011	KBr	0.04 M					1	dodecyl pyridinium bromide
		+	25	0.0049	LiBr	0.06 M					4	
		+	25	0.004	RbBr	0.06 M					4	
		+	25	0.00335	KBr	0.08 M					4	
		+	25	0.0034							4	
C17H30CIN	284	+	25	0.0147							4	dodecylpyridinium chloride
			25	0.0124	NaCl	0.01M					1	
			25	0.0045	NaCl	0.1M					1	
C17H35NaO3S	342	-	50	0.0002							1	sodium heptadecylsulfonate
C17H35NaO4S	358	-	40	0.0023							1	sodium heptadecyl 9-sulfate
C17H35NaO4S	358	-	40	0.0005							1	sodium heptadecyl 2-sulfate
C17H38BrN	336	+	20	0.0034		10%v/v						tetradecyltrimethylammonium bromide
			20	0.0042	EtOH	25%v/v					1	
			25	0.0035	EtOH						1	
			25	0.0014	NaBr	0.01M	0.336	72			2	
			25	0.0004	NaBr	0.08M					1	
			60	0.0045							1	
C17H38CIN	292	+	25	0.00447								tetradecyltrimethylammonium chloride
			25	0.00041	NaCl	0.1 M		65			4	
C17H38N2O3	318	+	25	0.0027							1	tetradecyltrimethylammonium nitrate

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C17H39NO	273	+	25	0.0045				42			1	tetradecyltrimethylammonium hydroxide
C17H39NO4P	352	Z	20	0.0013							7	dodecyl trimethylammonioethyl phosphate or dodecyl phosphocholine
C18H29NO2	291	Z	25	0.0015			0.276	70	12		8	N-dodecylpyridinio meta carboxylate
C18H29NO2	291	Z	50	0.0019			0.276	70	42		8	N-dodecylpyridinio para carboxylate
C18H29NaO3S	348	-	20	0.0023				21			1	sodium dodecyl 6-benzene sulfonate
C18H29NaO3S	348	-	20	0.002				64	14		1	sodium dodecyl 3-benzene sulfonate
C18H29NaO3S	348	-	25	0.002							1	sodium dodecyl 2-benzene sulfonate
C18H29NaO3S	348	-	25	0.0016				24			1	sodium dodecyl 4-benzene sulfonate
C18H29NaO3S	348	-	60	0.0012				103	52		4	sodium dodecylbenzene sulfonate
C18H30O3	294	N	25	0.000077							4	p-octylbenzene polyoxyethylene2 alcohol
C18H32BrN	344	+	30	0.0046							4	tridecyl pyridinium bromide
C18H35KO2	322	-	25	0.0005						0.58	2	potassium octadecanoate
C18H35NaO2	306	-	20	0.00028						0.58	2	sodium octadecanoate
C18H37NO2	299	Z	25	0.00032							9	tetradecyl betaine
C18H37NaO3S	356	-	60	0.00019			0.367				1	sodium octadecylsulfonate
C18H37NaO4S	372	-	40	0.00016					40.5	0.46	2	sodium octadecylsulfate
C18H37NaO4S	372	-	40	0.00072							1	sodium octadecyl 6-sulfate
C18H37NaO4S	372	-	40	0.00026							1	sodium octadecyl 2-sulfate
C18H37NaO4S	372	-	40	0.00045							1	sodium octadecyl 4-sulfate
C18H37NaO4S	372	-	60	0.0002						0.46	2	sodium octadecylsulfate
C18H38O4	318	N	25	0.0001			0.32		<0		6	polyoxyethylene3 dodecyl ether
C18H38O5	334	N	25	0.00081			0.335		21		6	polyoxyethylene4 decyl ether
C18H38O6	349	N	25	0.00019							4	dodecyl beta D-glucoside

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C18H38O7	366	N	20	0.1							3	polyoxyethylene6 butyl(2-ethyl) ether
C18H38O7	366	N	20	0.074					83		3	polyoxyethylene6 hexyl ether
C18H40ClN	306	+	25	0.00025							2	octadecylammonium chloride
C19H34BrN	356	+	20	0.00285				79		0.72	10	tetradecylpyridinium bromide
			20	0.00285	NaBr	0.0001 M				0.74	10	
			20	0.00255	NaBr	0.001 M				0.76	10	
			20	0.0016	NaBr	0.005 M				0.8	10	
			20	0.0011	NaBr	0.01 M				0.8	10	
			20	0.00075	NaBr	0.02 M		113		0.76	10	
			30	0.0024							2	
C19H39NaO4S	386	-	60	0.00092							1	sodium nonadecyl 10-sulfate
C19H39NaO4S	386	-	60	0.00033							1	sodium nonadecyl 5-sulfate
C19H42BrN	364	+	20	0.00089							2	hexadecyltrimethylammonium bromide CTAB
			20	0.0003	BuOH	4% v/v					1	
			20	0.0001	BuOH	6.5% v/v					1	
			25	0.00083			0.364	90	26	0.84	2	
			25	0.00054	NaBr	0.001M					1	
			25	0.0001	NaBr	0.01M					1	
			25	0.00049	NaCl	0.01M					1	
			25	0.00023	NaCl	0.1M					1	
			25	0.00069	EtOH	15%v/v				0.9	5	
			25	0.0011	EtOH	25%v/v					1	
			25	0.00083	MeOH	5%v/v		70		0.8	5	
			35	0.00102							1	
			45	0.00116							1	

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
			55	0.00132							1	
			60	0.0013			0.364				2	
C19H42CIN	320	+	25	0.0013							1	hexadecyltrimethylammonium chloride
			25	0.0001	NaCl	0.1M		81			2	CTACl
			25	0.00095	EtOH	15%v/v		136			1	
			25	0.0011	EtOH	25%v/v					1	
C19H42N2O3	346	+	25	0.00081							1	hexadecyltrimethylammonium nitrate
			25	0.00081	EtOH	15%v/v					1	
C19H43NO	301	+	25	0.0018				46			1	hexadecyltrimethylammonium hydroxide
C19H43NO3S	366	M	25	0.0202							4	octyl trimethylammonium octane sulfonate
C19H43NO4P	380	Z	20	0.00012							7	tetradecyl trimethylammonioethyl phosphate or tetradecyl phosphocholine
C19H43NO4S	382	M	25	0.0075							4	octyl trimethylammonium octyl sulfate
C20H29KO2	350	-	25	0.00013						0.58	2	potassium eicosanoate
C20H29NaO2	334	-	20	0.0001						0.58	2	sodium eicosanoate
C20H33NaO3S	376	-	25	0.0012							1	sodium 2-undecyl 5-propyl benzenesulfonate
C20H33NaO3S	376	-	25	0.00072							1	sodium 2-propyl 5-undecyl benzenesulfonate
C20H33NaO3S	376	-	25	0.00066							1	sodium 2-tridecyl 5-methyl benzenesulfonate
C20H33NaO3S	376	-	25	0.0021							1	sodium 2,5-diheptyl benzenesulfonate

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C20H33NaO3S	376	-	25	0.00088							<i>1</i>	sodium 2-dodecyl 5-ethyl benzenesulfonate
C20H33NaO3S	376	-	25	0.0005							<i>1</i>	sodium 2-ethyl 5-dodecyl benzenesulfonate
C20H33NaO3S	376	-	70	0.0005				122	59		<i>1</i>	sodium tetradecylbenzene sulfonate
C20H34O4	338	N	25	0.0001			0.393				<i>4</i>	p-octylbenzene polyoxyethylene3 alcohol
C20H36ClN	326	+	25	0.0078							<i>4</i>	dodecyl benzyl dimethylammonium chloride
C20H37NO3S	371	-	20	0.0017				26			<i>1</i>	dimethyl ammonium dodecyl 6-benzene sulfonate
C20H37NaO7S	445	-	25	0.0025							<i>4</i>	sodium di2-ethyl hexyl sulfosuccinate, AOT
C20H37NaO7S	445	-	25	0.00068							<i>4</i>	sodium dioctyl sulfosuccinate
C20H39NaO4S	398	-	20	0.0003							<i>11</i>	sodium phytolsulfate
C20H40NaO4P	398	-	20	0.0013							<i>11</i>	sodium phytolphosphate
C20H41NO2	327	Z	25	0.0001							<i>9</i>	hexadecyl betaine
C20H41NaO4S	400	-	45	0.000032					45	0.46	<i>2</i>	sodium eicosylsulfate
			60	0.000056						0.46	<i>1</i>	
C20H42MgO8S2	467	-	60	0.02							<i>4</i>	magnesium decylsulfonate
C20H42O5	362	N	25	0.000065			0.36		5		<i>6</i>	polyoxyethylene4 dodecyl ether
C20H42O6	390	N	25	0.00082			0.39		44		<i>6</i>	polyoxyethylene5 decyl ether
C20H42O7	394	N	25	0.01			0.394	33	71		<i>4</i>	polyoxyethylene6 octyl ether
C20H44BrNO	394	+	25	0.0104							<i>11</i>	(6-hydroxyhexyl)dodecyl dimethyl ammonium bromide
C20H44ClN	334	+	25	0.0001							<i>2</i>	eicosylammonium chloride
C20H45NO4S	395	-	30	0.0045							<i>3</i>	tetraethylammonium dodecylsulfate

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C21H38BrN	384	+	20	0.0048							10	dodecylbenzyltrimethylammonium bromide
C21H38BrN	384	+	30	0.00057					25		2	hexadecylpyridinium bromide
C21H38CIN	340	+	25	0.0006					11		4	hexadecylpyridinium chloride
			25	0.00016	NaCl	0.01M		95			1	
			25	0.000038	NaCl	0.1M		135			1	
			80	0.00236							4	
C21H39NaO2	346	-	20	0.0016							11	sodium phytolcarboxylate
C21H46BrN	392	+	25	0.00021							2	octadecyltrimethylammonium bromide
			60	0.00035							2	
C21H46CIN	348	+	25	0.0004		6% v/v					4	octadecyltrimethylammonium chloride
			25	0.000020	MeOH NaCl	0.1 M					2	
C21H47NO4P	408	Z	20	0.000032							7	hexadecyl trimethylammonioethyl phosphate or hexadecyl phosphocholine
C22H37NaO3S	404	-	75	0.00054				130	49		1	sodium hexadecylbenzene sulfonate
C22H38O5	382	N	25	0.00013							4	p-octylbenzene polyoxyethylene4 alcohol
C22H41NO3S	399	-	20	0.0014				30			1	diethyl ammonium dodecyl 6-benzene sulfonate
C22H45NaO4S	428	-	60	0.000014						0.46	1	sodium docosylsulfate
C22H46O6	406	N	25	0.00006			0.41		26		1	polyoxyethylene5 dodecyl ether
C22H46O7	422	N	20	0.0031							3	polyoxyethylene6 hexyl(2-butyl) ether
C22H46O7	422	N	25	0.00083			0.422	73	60		2	polyoxyethylene6 decyl ether

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C22H48BrNO	422	+	25	0.0052							12	(8-hydroxyoctyl)dodecyldimethyl ammonium bromide
C23H42BrN	412	+	20	0.0026							10	tetradecyldimethylbenzylammonium bromide
C23H42BrN	412	+	30	0.00014							2	octadecylpyridinium bromide
C23H42ClN	368	+	20	0.0021							10	tetradecyldimethylbenzylammonium chloride
C23H48BrN	415	+	20	0.0014							11	phytyltrimethylammonium bromide
C23H50BrN	420	+	25	0.0001							2	eicosyltrimethylammonium bromide
C23H50BrN	420	+	60	0.0001							2	eicosyltrimethylammonium bromide
C23H50ClN	376	+	25	0	NaCl	0.1 M					2	eicosyltrimethylammonium chloride
C23H51NO3S	422	M	25	0.00136							4	decyl trimethylammonium decane sulfonate
C23H51NO4S	438	M	25	0.00046							4	decyl trimethylammonium decyl sulfate
C24H41NaO3S	432	-	75	0.00064				123	58		1	sodium octadecylbenzene sulfonate
C24H42O6	426	N	25	0.00015			0.495				4	p-octylbenzene polyoxyethylene5 alcohol
C24H45NO3S	427	-	40	0.0012				24			1	dipropyl ammonium dodecyl 6-benzene sulfonate
C24H50CaO6S2	571	-	70	0.0034							3	calcium dodecylsulfate
C24H50MgO6S2	523	-	60	0.0036							4	magnesium dodecylsulfonate
C24H50O7	451	N	25	0.000087			0.45	400	50		2	polyoxyethylene6 dodecyl ether
			25	0.000138	EtOH	10%v/v					1	
			25	0.000094	EtOH	5%v/v					1	

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C25H44O6	440	N	25	0.000032							1	p-nonylbenzene polyoxyethylene5 alcohol
C25H46BrN	440	+	30	0.000033							2	eicosylpyridinium bromide
C25H52O9	496	N	25	0.003			0.496		95		1	polyoxyethylene8 nonyl ether
C25H54ClN	404	+	20	0.0019							10	trioctylmethylammonium bromide
C26H45NaO3S	460	-	55	0.000076				352			2	sodium eicosylbenzene sulfate
C26H46O7	470	N	25	0.000205							4	p-octylbenzene polyoxyethylene6 alcohol
C26H54O10	526	N	25	0.013			0.526		~100		4	polyoxyethylene9 octyl ether
C26H54O7	478	N	25	0.0000091				3000	42		2	polyoxyethylene6 tetradecyl ether
C26H54O8	494	N	25	0.000080			0.494		65		6	polyoxyethylene7 dodecyl ether
			25	0.00013	urea	6 M					1	
C26H54O9	510	N	25	0.001			0.51		84		1	polyoxyethylene8 decyl ether
			25	0.0026	NaCholate	0.0019 M					1	
			25	0.01	NaCholate	0.01 M					1	
C26H56BrNO	478	+	35	0.0013							12	(12-hydroxydodecyl)dodecyltrimethyl ammonium bromide
C27H56O9	524	N	25	0.0003			0.524		82		1	polyoxyethylene8 undecyl ether
C28H50O8	514	N	25	0.00025			0.598		25		4	p-octylbenzene polyoxyethylene7 alcohol
C28H58O10	554	N	20	0.0032							3	polyoxyethylene9 hexyl(2-butyl) ether
C28H58O10	555	N	25	0.0013			0.535		90		4	polyoxyethylene9 decyl ether
C28H58O7	506	N	25	0.0000010				2350	32		2	polyoxyethylene6 hexadecyl ether
C28H58O9	538	N	25	0.00008			0.538	126	80		6	polyoxyethylene8 dodecyl ether

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C29H59NaO4S	526	-	40	0.00008						0.46	1	sodium nonacosyl 10-sulfate
C29H60O9	552	N	25	0.00003				70	72		1	polyoxyethylene8 tridecyl ether
C30H54O9	558	N	25	0.00028					40		4	p-octylbenzene polyoxyethylene8 alcohol
C30H62O10	582	N	25	0.0001			0.582		88		1	polyoxyethylene9 dodecyl ether
C30H62O7	534	N	25	0.0000001							2	polyoxyethylene6 octadecyl ether
C30H62O8	550	N	25	0.0000017					53		4	polyoxyethylene7 hexadecyl ether
C30H62O8	550	N	25	0.0000090					70		1	polyoxyethylene8 tetradecyl ether
C31H64O8	564	N	25	0.0000035					66		1	polyoxyethylene8 pentadecyl ether
C32H58O10	602	N	25	0.00027				111	54		4	p-octylbenzene polyoxyethylene9 alcohol
C32H58O9.5	594	N	25	0.0001					68		1	p-nonylbenzene polyoxyethylene 8.5 alcohol, Igepal® C610
C32H66O7	562	N	25	0.00000001							2	polyoxyethylene6 eicosyl ether
C32H66O9	594	N	25	0.0000020				240	65		1	polyoxyethylene 8 hexadecyl ether
C33H60O10	616	N	25	0.0001				350	51		1	p-nonylbenzene polyoxyethylene 9 alcohol
C33H60O10.5	624	N	25	0.0003			0.743	140	67		1	p-octylbenzene polyoxyethylene 9.5 alcohol, Triton® X100
			25	0.00027	NaCl	0.001 M		140			1	
			25	0.00034	NaCl	0.1 M		135			1	
			25	0.00026	KI	0.36 M		122			1	
			25	0.00025	KBr	0.36 M		102			1	
C33H68O11	640	N	25	0.00012				61			1	polyoxyethylene 10 tridecyl ether

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C34H62O10.5	638	N	25	0.000085			0.574	265			1	p-nonylbenzene polyoxyethylene 9.5 alcohol, Igepal® CO 630
C34H70O10	639	N	25	0.0000021				220	75		4	polyoxyethylene 9 hexadecyl ether
C35H64O11	660	N	25	0.0001	NaCl dioxane urea	0.03 M 25% v/v 6 M	0.602	150 120			1	p-nonylbenzene polyoxyethylene 10 alcohol, Igepal® CO 660
			25	0.0001							1	
			25	0.00018							1	
			25	0.00024							1	
C36H66O11.5	682	N	25	0.0001			0.614	125			1	p-nonylbenzene polyoxyethylene 10.5 alcohol, Igepal® CO 710
C36H74O13	714	N	25	0.00014			0.714	83	100>		1	polyoxyethylene 12 dodecyl ether
C37H76O13	728	N	25	0.0002				55			1	polyoxyethylene 12 tridecyl ether
C38H84N2O4S	664	+	25	0.0006							1	hexadecyltrimethylammonium sulfate
C39H84N2O5	628	+	25	0.0008							1	hexadecyltrimethylammonium carbonate
C40H74O14	778	N	25	0.00028			0.905		87		1	p-octylbenzene polyoxyethylene 13 alcohol
C40H82O13	770	N	25	0.0000023				160	92		4	polyoxyethylene 12 hexadecyl ether
C40H82O15	802	N	25	0.0000550			0.802		100>		3	polyoxyethylene 14 dodecyl ether
C43H87NO4P	712	M	20	0.0000049							11	phytyl trimethylammonium phytyl phosphate

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C43H87NO4S	713	M	20	0.0000053							11	phytyl trimethylammonium phytyl sulfate
C44H87NO2	661	M	20	0.0000077							11	phytyl trimethylammonium phytyl carboxylate
C45H84O16	880	N	25	0.00011	NaCl	1.3M	0.792	80			1	p-nonylbenzene polyoxyethylene 15 alcohol, Igepal® CO 730
			25	0.0000450							1	
C46H86O17	910	N	25	0.00043			1.006				1	p-octylbenzene polyoxyethylene 16 alcohol
C46H94O31	902	N	25	0.0000031					100>		4	polyoxyethylene 15 hexadecyl ether
C48H90O18	954	N	25	0.00034			1.129				1	p-octylbenzene polyoxyethylene 17 alcohol
C48H98O19	978	N	25	0.00026			0.98	51	100>		1	polyoxyethylene 18 dodecyl ether
C55H104O21	1100	N	25	0.00014			0.971	60			1	p-nonylbenzene polyoxyethylene 20 alcohol, Igepal® CO 850
C58H118O22	1166	N	25	0.0000039				70	100>		4	polyoxyethylene 21 hexadecyl ether
C58H118O24	1198	N	25	0.000060	EtOH dioxane dioxane EtOH	10%v/v	1.12	41	100>		3	polyoxyethylene 23 dodecyl ether Brij® 35
			25	0.0012		10%v/v					1	
			25	0.0016		20%v/v					1	
			25	0.003		20%v/v					1	
			25	0.0024							1	
C72H146O31	1506	N	25	0.00008	urea	6M	1.5		100>		1	polyoxyethylene 30 dodecyl ether
			25	0.00025							1	
C74H150O32	1550	N	25	0.00008					100>		1	polyoxyethylene 31 dodecyl ether
C75H144O31	1540	N	25	0.0017			1.361	60	100>		1	p-nonylbenzene polyoxyethylene 30 alcohol, Igepal® CO 880

Surfactant	mw dalt.	charge	temp °C	CMC M	medium		molar vol. L/mole	aggr. numb	Kr. or Cl. pt. °C	counter ion binding	ref	name
					additive	conc. M or % v/v						
C76H154O31	1562	N	25	0.000011	urea	6M			100>		/	polyoxyethylene 30 hexadecyl ether
			25	0.000020								
C77H148O32	1584	N	25	0.00018	dioxane urea	25%v/v 6M	1.426		100>		/	p-nonylbenzene polyoxyethylene 31 alcohol
			25	0.00057								
			25	0.00074								
C88H170O41	1882	N	25	0.00081					100>		/	p-octylbenzene polyoxyethylene 40 alcohol
C95H184O41	1980	N	25	0.00019			1.782	25	100>		/	p-nonylbenzene polyoxyethylene 40 alcohol, Igepal® CO 890
C115H224O51	2420	N	25	0.00025	NaCl	1.3M	2.125	20	100>		/	p-nonylbenzene polyoxyethylene 50 alcohol, Igepal® CO 970
			25	0.0001								
C215H424O101	4620	N	25	0.001					100>		/	p-nonylbenzene polyoxyethylene 100 alcohol

MeOH = methanol; EtOH = ethanol; PrOH = propanol; BuOH = butanol; C5OH = pentanol; C6OH = hexanol; C7OH = heptanol; phytol = 2,6,10,14-tetramethyl-14-hexadecenyl (C20H39).

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Appendix III

Surfactant Affinity Coefficients

Exposed in several chapters is the fact that a strong point of Micellar Liquid Chromatography (MLC) is its capability to give the micellar partition coefficient of the analyzed solute. The Armstrong-Nome equation was presented in Chapters 3 and 5, Equations 3.4 and 5.1. The variations of this equation were also exposed (Eqs. 5.8 and 5.9).

Chapter 5 discusses the unit problem that exists in the literature listing micellar partition coefficients. The Armstrong-Nome equation gives the P_{WM} solute-micelle affinity coefficient. It is a dimensionless coefficient but valid for one surfactant molecule. The true solute micellar partition coefficient is $P_{WM} \times N$ with N the micelle aggregation number. Equations 5.8 and 5.9 give K_{AM} , an equivalent coefficient whose dimension depends on the concentration unit used. All equations relate linearly $1/k$, the retention factor, with $[M]$, the micellar concentration (= surfactant concentration - cmc) in mol/L or in g/L. The relations between the two coefficients are:

$$K_{AM} = \text{slope/intercept} = v (P_{WM} - 1) \quad (\text{III.1})$$

or

$$P_{WM} = K_{AM}/v + 1 \quad (\text{III.2})$$

The possible units for v , the surfactant molar volume, are g/L or mol/L. To add to the confusion, the molar volume of CTAB is 0.999 g/mL. This means

that the value of the CTAB P_{WM} coefficient is numerically the same as the corresponding K_{AM} value expressed in g/mL. The v value of SDS is 0.854 mL/g producing K_{AM} values numerically 15% lower than the corresponding P_{WM} values. This 15% difference is within the error margin obtained in such coefficient determination (accuracy $\sim 20\%$).

The solute affinity for the surfactant covered stationary phase is expressed by ϕP_{WS} or K_{AS} . These two coefficients are identical. Since the phase ratio, ϕ , is rarely known, the dimensionless product ϕP_{WS} is listed. The following table was prepared after reviewing original articles. If the slopes and intercepts of the $1/k$ versus $[M]$ lines were given, the K_{AM} value was calculated according to Eq. III.1 and the ϕP_{WS} value was taken as $1/\text{intercept}$. Then P_{WM} and the other K_{AM} values were calculated using Eqs. III.1 and III.2. A modern data processor was used producing 12 digits in all calculations. Due to the seven orders of magnitude range of the P or K values, it was not possible to round these numbers correctly. Only the first two digits are significant, e.g. the listed P_{WM} value of 956 in CTAB for dinitrophenol should be read as 960 ± 190 or $\log P_{WM} = 3.0 \pm 0.06$. The dinitrophenol micellar partition coefficient, P , can be estimated as 86500 since the CTAB aggregation number is 90 (Appendix II) or $\log P = 4.9 \pm 0.06$. The aggregation number changes if any modifier is added to the micellar phase (see Chapter 2 and Appendix II). In a lower extent, the molar volumes are also affected by the modifiers. However, the v values in aqueous micellar phase were used throughout the table for calculations.

The table is first sorted by global solute formula, then by alphabetic order of the isomers and finally by surfactant in the mobile phase. The 11 columns give successively: 1) the global solute formula, 2) its name, 3) the stationary phase used, 4) the surfactant used in the micellar phase, 5) the additive in the micellar phase, 6) the temperature, 7) the dimensionless P_{WM} value, 8) the K_{AM} value in L/mol, 9) the K_{AM} value in mL/g, 10) the dimensionless K_{AS} value or the ϕK_{WS} value and 11) the reference. The reference titles were omitted to save space and because they are already listed in Chapter 5 and elsewhere in the book.

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ϕP_{WS} or K_{AS}	ref.
Br-	ion bromure	Spherisorb ODS - 5 μ m	CTACl			2842	889	2776		1
C2H6O	ethanol	Shein-Pack diol GPC	SDS		40	3	0.5	1.7	0	2
C3H8O	propanol	Cellulofine Gpc-400	SDS		25	5	1.0	3.5		2
C4H10O	butanol	Cellulofine Gpc-400	SDS		25	22	5.1	18		2
C4H6N4O3S2	acetazolamide	Spherisorb ODS-2 C18 - 5 μ m	SDS			19	4.5	16	2.1	3
C5H12O	pentanol	Cellulofine Gpc-100	SDS		25	85	20.5	72		2
C5H11NO2S	methionine	Bakerbond C18	SDS		40	69	17	58		4
C6Cl5O	pentachlorophenol	Bakerbond C18	SDS		40	25	5.9	20		4
C6Cl5O	pentachlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	134	33	114	162	5
C6H3Cl3O	2,4,5-trichlorophenol	Bakerbond C18	SDS		40	138	34	117		4
C6H3Cl3O	2,4,5-trichlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	95	23	81	78	5
C6H3Cl3O	2,4,6-trichlorophenol	Bakerbond C18	SDS		40	30	7.0	24		4
C6H3Cl3O	2,4,6-trichlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	80	19	68	62	5
C6H4Cl2O	2,3-dichlorophenol	Bakerbond C18	SDS		40	81	20	69		4
C6H4Cl2O	2,3-dichlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	51	12	43	29	5
C6H4Cl2O	2,4-dichlorophenol	Rainin Microsorb C18 3 μ m	CTAB		35	3847	1400	3842	2703	6
C6H4Cl2O	2,4-dichlorophenol	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	570	140	486	143	6
C6H4Cl2O	2,4-dichlorophenol	Bakerbond C18	SDS		40	97	24	82		4
C6H4Cl2O	2,4-dinitrophenol	Rainin Microsorb C18 3 μ m	CTAB		35	963	350	962	476	6
C6H4Cl2O	2,5-dichlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	49	12	41	31	5
C6H4Cl2O	2,5-dichlorophenol	Bakerbond C18	SDS		40	60	15	51		4
C6H4Cl2O	3,5-dichlorophenol	Bakerbond C18	SDS		40	122	30	103		4

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C6H5Br	bromobenzene	Rainin Microsorb C18 3 μ m	CTAB		35	474	172	472	256	6
C6H5Br	bromobenzene	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	428	105	365	222	6
C6H5BrO	bromophenol	Rainin Microsorb C18 3 μ m	CTAB		35	655	238	653	345	6
C6H5BrO	bromophenol	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	131	32	111	27	6
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	Brij-35		25	285	302	252	312	7
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB		25	1506	548	1504	690	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	3941	1434	3936	1614	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	1188	432	1185	488	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	644	234	642	228	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	366	133	365	122	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	151	54	149	52	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	78	28	77	20	8
C6H5Cl	chlorobenzene	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	74	27	73	164	11
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	CTAB		25	440	160	438	192	7
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	CTAB	5% BuOH	25	248	90	247	83	9
C6H5Cl	chlorobenzene	Rainin Microsorb C18 3 μ m	CTAB		35	284	103	283	154	6
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS		25	429	105	366	210	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	245	60	208	93	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	296	72	252	109	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	253	62	215	104	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	227	56	193	67	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	207	51	176	66	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ΦP_{WS} or K_{AS}	ref.
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	116	28	99	44	8
C6H5Cl	chlorobenzene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	72	18	61	16	8
C6H5Cl	chlorobenzene	Altex ODS	SDS	3% 2-PrOH	38	68	16	57	227	11
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	SDS		25	279	68	238	169	7
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	SDS	10% BuOH	25	188	46	160	40	9
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	SDS	5% BuOH	25	221	54	187	77	9
C6H5Cl	chlorobenzene	Novapack C18 4 μ m	SDS	NaCl 0.1M	25	334	82	285	130	9
C6H5Cl	chlorobenzene	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	294	72	250	147	6
C6H5ClO	2-chlorophenol	Bakerbond C18	SDS		40	26	6.2	21		4
C6H5ClO	2-chlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	18	4.2	15	8.1	3
C6H5ClO	3-chlorophenol	Bakerbond C18	SDS		40	38	9.0	31		4
C6H5ClO	3-chlorophenol	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	27	6.5	23	14	5
C6H5ClO	4-chlorophenol	Bakerbond C18	SDS		40	41	10	34		4
C6H5ClO	o-chlorophenol	Rainin Microsorb C18 3 μ m	CTAB		35	489	120	417	172	6
C6H5ClO	o-chlorophenol	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	90	22	76	18	6
C6H5ClO2	2-chlorobenzene-1,4-diol	μ Bondapak C18	SDS		24	34	8.6	30	3.7	10
C6H5NO2	nitrobenzene	Novapack C18 4 μ m	Brij-35		25	60	62	52	38	7
C6H5NO2	nitrobenzene	Spherisorb C8 - 5 μ m	CTAB		25	151	55	150	59	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	120	43	119	41	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	103	37	102	35	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	85	30	84	24	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	82	29	81	22	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	45	16	43	13	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	35	12	34	7.8	8
C6H5NO2	nitrobenzene	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	26	9.1	25	44	11
C6H5NO2	nitrobenzene	Novapack C18 4μm	CTAB		25	104	37	102	31	7
C6H5NO2	nitrobenzene	Novapack C18 4μm	CTAB	5% BuOH	25	50	18	49	11	9
C6H5NO2	nitrobenzene	Rainin Microsorb C18 3μm	CTAB		35	92	33	91	40	6
C6H5NO2	nitrobenzene	Supelcosil LC-1 5 μm	DTAB		25	2	23	1	34	12
C6H5NO2	nitrobenzene	Supelcosil LC-1 5 μm	SDS		25	2	22	1	37	12
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS		25	107	26	90	34	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	10% MeOH	25	90	22	76	21	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	3% n-PrOH	25	75	18	63	20	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	5% n-PrOH	25	65	16	55	18	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	10% n-PrOH	25	57	14	48	12	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	52	13	44	13	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	31	7.3	25	10	8
C6H5NO2	nitrobenzene	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	32	7.7	27	5.9	8
C6H5NO2	nitrobenzene	Altex ODS	SDS	3% 2-PrOH	38	22	5.2	18	34	11
C6H5NO2	nitrobenzene	Novapack C18 4μm	SDS		25	94	23	80	28	7
C6H5NO2	nitrobenzene	Bakerbond C18	SDS		40	23	5.5	19		4
C6H5NO2	nitrobenzene	Novapack C18 4μm	SDS	10% BuOH	25	62	15	52	7,0	9
C6H5NO2	nitrobenzene	Novapack C18 4μm	SDS	5% BuOH	25	46	11	38	9,0	9
C6H5NO2	nitrobenzene	Novapack C18 4μm	SDS	NaCl 0,1M	25	90	22	76	24	9
C6H5NO2	nitrobenzene	Rainin Microsorb C18 3μm	SDS	2% 1-PrOH	25	8	1,7	5,9	2,4	6

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C6H5NO2	nitrobenzene	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	15	3.5	12	10	5
C6H5NO3	p-nitrophenol	Rainin Microsorb C18 3 μ m	CTAB		35	468	170	467	233	6
C6H5NO3	p-nitrophenol	Supelcosil LC-1 5 μ m	DTAB		25	2	61	200	120	12
C6H5NO3	p-nitrophenol	Micropak C18 10 μ m	SDS		22	33	8.1	28	14	13
C6H5NO3	p-nitrophenol	Micropak CN 10 μ m	SDS		22	34	8.2	28	1.5	13
C6H5NO3	p-nitrophenol	Supelcosil LC-1 5 μ m	SDS		25	78	19	66	14	12
C6H5NO3	p-nitrophenol	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	62	15	52	7.7	6
C6H6	benzene	Supelcosil LC-18	Brij 30	60% ACN		40	19	51		14
C6H6	benzene	Supelcosil LC-18	Brij 30	60% ACN		40	19	51		14
C6H6	benzene	Radial-PAK C18 - 10 μ m	Brij-35			54	13	45	85	15
C6H6	benzene	Radial-PAK C18 - 10 μ m	Brij-35	15% EtOH						15
C6H6	benzene	Novapack C18 4 μ m	Brij-35		25	34	35	29	36	7
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB		25	131	47	130	55	8
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	131	47	130	49	8
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	126	46	125	47	8
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	104	37	103	34	8
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	102	37	101	31	8
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	64	23	63	21	8
C6H6	benzene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	49	17	48	12	8
C6H6	benzene	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	26	9.1	25	51	11
C6H6	benzene	Altex Octyl Ultrasphere	CTAB		31	67	24	66	52	16
C6H6	benzene	Novapack C18 4 μ m	CTAB		25	113	41	112	43	7
C6H6	benzene	Novapack C18 4 μ m	CTAB	5% BuOH	25	75	27	74	24	9

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ΦP_{WS} or K_{AS}	ref.
C6H6	benzene	Rainin Microsorb C18 3 μ m	CTAB		35	100	36	99	48	6
C6H6	benzene	Supelcosil LC-18	DOSS	39% ACN			15	35		14
C6H6	benzene	Supelcosil LC-18	DOSS	39% ACN			15	35		14
C6H6	benzene	Supelcosil LC-1 5 μ m	DTAB		25	69	21	68	27	12
C6H6	benzene	Hypersil ODS C18	MB-14				63	212	36	17
C6H6	benzene	Hypersil ODS C18	SB-12				34	101	34	17
C6H6	benzene	Supelcosil LC-1 5 μ m	SDS		25	78	19	66	23	12
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS		25	97	24	82	37	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	72	17	61	23	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	79	19	66	25	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	65	16	55	25	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	70	17	59	19	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	68	16	57	19	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	55	13	46	18	8
C6H6	benzene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	45	11	38	9	8
C6H6	benzene	Altex ODS	SDS	3% 2-PrOH	38	24	5.6	19	62	11
C6H6	benzene	μ Bondapak C-18 (Waters)	SDS		25	103	25	87	27	18
C6H6	benzene	Rainin Microsorb 5 μ m octyl	SDS		31	70	17	59	56	16
C6H6	benzene	Rainin Microsorb 5 μ m octyl	SDS	3% PrOH	31	66	16	56	43	16
C6H6	benzene	Rainin Microsorb 3 μ m - C18	SDS		31	86	21	73	82	16
C6H6	benzene	Novapack C18 4 μ m	SDS		25	79	19	67	38	7
C6H6	benzene	Novapack C18 4 μ m	SDS	10% BuOH	25	78	19	66	16	9
C6H6	benzene	Novapack C18 4 μ m	SDS	5% BuOH	25	66	16	56	21	9

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C6H6	benzene	Novapack C18 4 μ m	SDS	NaCl 0,1M	25	86	21	73	28	9
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS		35	54	13	45	23	19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS		25	84	20	70		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS		35	77	19	65		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS		45	75	18	63		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	62	15	52		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	60	15	51		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	58	14	49		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	57	14	48		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	55	13	46		19
C6H6	benzene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	51	12	43		19
C6H6	benzene	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	147	36	125	50	6
C6H6	benzene	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	17	3.9	14	16	5
C6H6	benzene	Supelcosil LC-18	THPA	60% ACN			13	27		14
C6H6	benzene	Supelcosil LC-18	Tween 60	60% ACN		194	242	184		14
C6H6N2O2	p-nitroaniline	Supelcosil LC-1 5 μ m	DTAB		25	148	45	147	53	12
C6H6N2O2	p-nitroaniline	Micropak C18 10 μ m	SDS		22	76	19	64	27	13
C6H6N2O2	p-nitroaniline	Micropak CN 10 μ m	SDS		22	81	20	69	3.4	13
C6H6N2O2	p-nitroaniline	Supelcosil LC-1 5 μ m	SDS		25	84	21	71	16	12
C6H6O	phenol	Supelcosil LC-18	Brij 30	60% ACN		27	13	35		14
C6H6O	phenol	Novapack C18 4 μ m	Brij-35		25	182	193	161	56	7
C6H6O	phenol	Inertsil ODS C18 - 5 μ m	Brij-35		25	57	62	50	37	20
C6H6O	phenol	Spherisorb C8 - 5 μ m	CTAB		25	219	80	218	83	8
C6H6O	phenol	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	167	60	166	49	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C6H6O	phenol	Spherisorb C8 - 5μm	CTAB	5% n-PrOH	25	105	38	103	30	8
C6H6O	phenol	Spherisorb C8 - 5μm	CTAB	10% n-PrOH	25	75	27	73	15	8
C6H6O	phenol	Spherisorb C8 - 5μm	CTAB	3% n-BuOH	25	64	23	63	17	8
C6H6O	phenol	Spherisorb C8 - 5μm	CTAB	5% n-BuOH	25	25	8.9	24	7.9	8
C6H6O	phenol	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	33	12	32	4.8	8
C6H6O	phenol	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	29	10	28	45	11
C6H6O	phenol	Novapack C18 4μm	CTAB		25	198	72	197	53	7
C6H6O	phenol	Novapack C18 4μm	CTAB	5% BuOH	25	48	17	47	8,0	9
C6H6O	phenol	Rainin Microsorb C18 3μm	CTAB		35	111	40	110	42	6
C6H6O	phenol	Supelcosil LC-18	DOSS	39% ACN			20	46		14
C6H6O	phenol	Supelcosil LC-1 5 μm	DTAB		25	94	30	1	34	12
C6H6O	phenol	Hypersil ODS C18	MB-14				142	477	125	17
C6H6O	phenol	Hypersil ODS C18	SB-12				162	484	139	17
C6H6O	phenol	μBondapak C18	SDS		24	40	10	35	7.7	10
C6H6O	phenol	Supelcosil LC-1 5 μm	SDS		25	38	9,0	1	8,0	12
C6H6O	phenol	Spherisorb C8 - 5μm	SDS		25	44	10.5	36	8.7	8
C6H6O	phenol	TSK-GPC 2000SW	SDS		25	38	9.1	32		2
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	10% MeOH	25	50	12.1	42	6.0	8
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	3% n-PrOH	25	39	9.4	33	5.8	8
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	5% n-PrOH	25	32	7.6	26	5.1	8
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	10% n-PrOH	25	37	8.8	31	3.9	8
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	28	6.6	23	3.9	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	25	5.9	21	3.8	8
C6H6O	phenol	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	27	6.4	22	2.5	8
C6H6O	phenol	Altex ODS	SDS	3% 2-PrOH	38	9	1.9	7	8.2	11
C6H6O	phenol	Novapack C18 4μm	SDS		25	39	9.4	33	7.2	7
C6H6O	phenol	Novapack C18 4μm	SDS	10% BuOH	25	50	12,0	42	3,0	9
C6H6O	phenol	Novapack C18 4μm	SDS	5% BuOH	25	25	6,0	21	3,0	9
C6H6O	phenol	Novapack C18 4μm	SDS	NaCl 0,1M	25	42	10,0	35	7,0	9
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS		35	29	7,0	24	4,0	19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS		25	39	9.5	33		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS		35	35	8.4	29		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS		45	32	7.6	26		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	32	7.7	27		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	32	7.6	26		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	30	7.0	24		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	31	7.4	26		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	29	6.9	24		19
C6H6O	phenol	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	26	6.1	21		19
C6H6O	phenol	Rainin Microsorb C18 3μm	SDS	2% 1-PrOH	25	29	7,0	24	5.0	6
C6H6O	phenol	Supelcosil LC-18	Tween60	60% ACN		109	135	103		14
C6H6O2	benzene-1,2-diol	μBondapak C18	SDS		24	27	6.9	24	5.0	10
C6H6O2	catechol	Rainin Microsorb C18 3μm	CTAB		35	105	38,0	104	35.7	6
C6H6O2	catechol	RP-18 (Waters) - 10μm	SDS		amb	25	6,0	20	5,0	22
C6H6O2	catechol	Rainin Microsorb C18 3μm	SDS	2% 1-PrOH	25	192	47,0	163	2.8	6

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C6H6O2	hydroquinone	Hypersil ODS C18	MB-14				31.3	105	25.6	17
C6H6O2	hydroquinone	Hypersil ODS C18	SB-12				207	616	98.0	17
C6H6O2	hydroquinone	Micropak C18 10µm	SDS		22	15	3.6	12	1.8	13
C6H6O2	hydroquinone	µBondapak C18	SDS		24	14	3.5	12	1.3	10
C6H6O2	hydroquinone	RP-18 (Waters) - 10µm	SDS		amb	17	4,0	14	3,0	22
C6H6O2	resorcinol	Inertsil ODS C18 5µm	Brij-35		25	59	65	52	25	20
C6H6O2	resorcinol	Rainin Microsorb C18 3µm	CTAB		35	100	36	99	28	6
C6H6O2	resorcinol	Hypersil ODS C18	MB-14				226	756	195	17
C6H6O2	resorcinol	Hypersil ODS C18	SB-12				138	410	118	17
C6H6O2	resorcinol	Micropak C18 10µm	SDS		22	28	6.8	23.4	5.2	13
C6H6O2	resorcinol	Micropak CN 10µm	SDS		22	26	6.3	21.8	0.9	13
C6H6O2	resorcinol	RP-18 (Waters) - 10µm	SDS		amb	21	5,0	18,0	4,0	22
C6H6O3	resorcinol	TSK-GPC 2000SW	SDS		25	17	4.0	14.0		2
C6H6O2	resorcinol	Rainin Microsorb C18 3µm	SDS	2% 1-PrOH	25	17	4,0	13,9	0.9	6
C6H7N	benzylamine	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	47	16.7	45.9	22.3	11
C6H7N	benzylamine	Altex ODS	SDS	3% 2-PrOH	38	16	3.7	12.7	12.4	11
C6H14O	hexanol	Cellulofine GPC-100	SDS		25	540	13.2	460		2
C7H5ClN3O4S2	chlorthiazide	Spherisorb ODS-2 5µm	SDS	Phosph. Buf.	20	53	12.9	44.7	0.5	23
C7H5N	benzonitrile	Novapack C18 4µm	Brij-35		25	16	16.2	13.5	13.3	7
C7H5N	benzonitrile	Spherisorb C8 - 5µm	CTAB		25	76	27.2	74.6	28.0	8
C7H5N	benzonitrile	Spherisorb C8 - 5µm	CTAB	3% n-PrOH	25	69	24.7	67.8	21.6	8
C7H5N	benzonitrile	Spherisorb C8 - 5µm	CTAB	5% n-PrOH	25	54	19.2	52.7	16.9	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C7H5N	benzonitrile	Spherisorb C8 - 5μm	CTAB	10% n-PrOH	25	47	16.8	46.2	12.4	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	CTAB	3% n-BuOH	25	58	20.7	56.9	13.5	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	CTAB	5% n-BuOH	25	25	8.8	24.2	7.7	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	26	9.0	24.7	5.4	8
C7H5N	benzonitrile	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	26	9.2	25.2	43.5	11
C7H5N	benzonitrile	Novapack C18 4μm	CTAB		25	55	19.7	53.9	16.1	7
C7H5N	benzonitrile	Novapack C18 4μm	CTAB	5% BuOH	25	28	10,0	27,4	6,0	9
C7H5N	benzonitrile	Rainin Microsorb C18 3μm	CTAB		35	50	18,0	49,3	20,0	6
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS		25	84	20.4	70.8	22.2	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	10% MeOH	25	83	20.2	70.2	15.3	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	3% n-PrOH	25	59	14.2	49.2	13.3	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	5% n-PrOH	25	46	11.1	38.4	10.6	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	10% n-PrOH	25	44	10.6	36.8	7.6	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	38	9.0	31.2	8.2	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	27	6.4	22.3	64.9	8
C7H5N	benzonitrile	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	30	7.2	25.1	4.4	8
C7H5N	benzonitrile	Altex ODS	SDS	3% 2-PrOH	38	16	3.7	13.0	19.6	11
C7H5N	benzonitrile	Novapack C18 4μm	SDS		25	135	32.9	114.1	27.0	7
C7H5N	benzonitrile	Novapack C18 4μm	SDS	10% BuOH	25	50	12,0	41,7	4,0	9
C7H5N	benzonitrile	Novapack C18 4μm	SDS	5% BuOH	25	34	8,0	27,8	5,0	9
C7H5N	benzonitrile	Novapack C18 4μm	SDS	NaCl 0,1M	25	90	22,0	76,4	18,0	9

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{ws} or K _{AS}	ref.
C7H5N	benzonitrile	Rainin Microsorb C18 3µm	SDS	2% 1-PrOH	25	50	12,0	41,7	14.3	6
C7H5N	benzonitrile	Nucleosil C18 5µm	SDS	3% 2-PrOH	40	12	2.7	9.3	5.9	5
C7H5NO2	4-cyanobenzene-1,2-diol	µBondapak C18	SDS		24	28	7.1	24.6	4.4	10
C7H6O	benzaldehyde	Waters Radial-PAK C18 - 10µm	Brij-35			18	4,2	15,0	21,0	15
C7H6O	benzaldehyde	Waters Radial-PAK C18 - 10µm	Brij-35	15% EtOH		15	3,5	12,0	12,0	15
C7H6O	benzaldehyde	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	15	5.1	13.9	22.7	11
C7H6O	benzaldehyde	Rainin Microsorb C18 3µm	CTAB		35	66	16,0	55,5	18.5	6
C7H6O	benzaldehyde	Altex ODS	SDS	3% 2-PrOH	38	17	3.9	13.7	21.0	11
C7H6O	benzaldehyde	Bakerbond C18	SDS		40	18	4.3	14.9		4
C7H6O	benzaldehyde	Rainin Microsorb C18 3µm	SDS	2% 1-PrOH	25	5,9	1,2	4,2	1.5	6
C7H6O	benzaldehyde	Nucleosil C18 5µm	SDS	3% 2-PrOH	40	11	2.5	8.7	5.8	5
C7H6O2	benzoic acid	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	53	18.9	51.9	84.0	11
C7H6O2	benzoic acid	Hypersil Shandon - 5µm	CTAB		amb	3300	1201	3296		24
C7H6O2	benzoic acid	CPS Hypersil Shandon - 5µm	CTAB		amb	2000	728	1997		24
C7H6O2	benzoic acid	SAS Hypersil Shandon - 5µm	CTAB		amb	1900	691	1897		24
C7H6O2	benzoic acid	MOS Hypersil Shandon 5µm	CTAB		amb	3200	1164	3196		24
C7H6O2	benzoic acid	ODS Hypersil Shandon 5µm	CTAB		amb	2800	1019	2796		24
C7H6O2	benzoic acid	SAS Hypersil Shandon - 5µm	CTAB		25	2765	680	2361	770	25
C7H6O2	benzoic acid	SAS Hypersil Shandon - 5µm	CTAB	NaCl -0.1M	25	692	170	590	220	25

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ΦP_{WS} or K_{AS}	ref.
C7H6O2	benzoic acid	SAS Hypersil Shandon - 5 μ m	CTAB	5% MeOH	25	3090	760	2638	900	25
C7H6O2	benzoic acid	ODS Hypersil Shandon 5 μ m	CTAB		25	4066	1000	3472	590	25
C7H6O2	benzoic acid	ODS Hypersil Shandon 5 μ m	CTAB	NaCl -0.1M	25	1078	265	920	190	25
C7H6O2	benzoic acid	ODS Hypersil Shandon 5 μ m	CTAB	5% MeOH	25	3172	780	2708	500	25
C7H6O2	benzoic acid	Altex ODS	SDS	3% 2-PrOH	38	10	2.3	8.0	5.0	11
C7H6O2	benzoic acid	ODS Hypersil Shandon 5 μ m	SDS		25	15	3	12	7	25
C7H6O2	benzoic acid	ODS Hypersil Shandon 5 μ m	SDS	5% MeOH	25	13	3	10	6	25
C7H6O2	p-hydroxybenzaldehyde	Inertsil ODS C18 5 μ m	Brij-35		25	53	58	46	26	20
C7H7NO	benzamide	Novapack C18 4 μ m	Brij-35		25	30	31	26	4	7
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB		25	35	12	34	9	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	29	10	28	6	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	22	7.7	21.0	4.6	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	20	7.0	19.1	3.0	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	24	8.2	22.6	4.3	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	16	5.5	15.2	2.9	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	9.4	3.0	8.3	1.6	8
C7H7NO	benzamide	Novapack C18 4 μ m	CTAB		25	32	11.5	31.4	4.7	7
C7H7NO	benzamide	Novapack C18 4 μ m	CTAB	5% BuOH	25	12	4.0	11.0	2.0	9
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	SDS		25	51	12.3	42.6	7.2	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	56	13.6	47.0	4.7	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	32	7.6	26.3	3.8	8
C7H7NO	benzamide	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	16	3.6	12.6	2.6	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C7H7NO	benzamide	Spherisorb C8 - 5μm	SDS	10% n-PrOH	25	20	4.6	16.0	1.8	8
C7H7NO	benzamide	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	20	4.6	16.1	2.2	8
C7H7NO	benzamide	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	10	2.2	7.5	1.7	8
C7H7NO	benzamide	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	18	4.2	14.6	1.1	8
C7H7NO	benzamide	Novapack C18 4μm	SDS		25	31	7.4	25.8	5.0	7
C7H7NO	benzamide	Novapack C18 4μm	SDS	10% BuOH	25	17	4,0	13,9	1,0	9
C7H7NO	benzamide	Novapack C18 4μm	SDS	5% BuOH	25	13	3,0	10,4	1,3	9
C7H7NO	benzamide	Novapack C18 4μm	SDS	NaCl 0,1M	25	58	14,0	48,6	8,0	9
C7H7NO3	p-nitroanisole	Rainin Microsorb C18 3μm	CTAB		35	245	60	208	71	6
C7H7NO3	p-nitroanisole	Rainin Microsorb C18 3μm	SDS	2% 1-PrOH	25	123	30	104	38	6
C7H8	toluene	Supelcosil LC-18	Brij-30	60% ACN		41	19	53		14
C7H8	toluene	Novapack C18 4μm	Brij-35		25	70	73	61	101	7
C7H8	toluene	Spherisorb C8 - 5μm	CTAB		25	561	204	560	261	8
C7H8	toluene	Spherisorb C8 - 5μm	CTAB	3% n-PrOH	25	773	281	772	322	8
C7H8	toluene	Spherisorb C8 - 5μm	CTAB	5% n-PrOH	25	560	203	558	236	8
C7H8	toluene	Spherisorb C8 - 5μm	CTAB	10% n-PrOH	25	349	127	348	129	8
C7H8	toluene	Spherisorb C8 - 5μm	CTAB	3% n-BuOH	25	287	104	286	95	8
C7H8	toluene	Spherisorb C8 - 5μm	CTAB	5% n-BuOH	25	126	45	125	45	8
C7H8	toluene	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	70	25	69	18	8
C7H8	toluene	Hypersil Shandon - 5μm	CTAB		amb	210	76	209		24

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C7H8	toluene	CPS Hypersil Shandon - 5 μ m	CTAB		amb	190	69	189		24
C7H8	toluene	SAS Hypersil Shandon - 5 μ m	CTAB		amb	280	102	279		24
C7H8	toluene	MOS Hypersil Shandon 5 μ m	CTAB		amb	360	131	359		24
C7H8	toluene	ODS Hypersil Shandon 5 μ m	CTAB		amb	390	142	389		24
C7H8	toluene	SAS Hypersil Shandon - 5 μ m	CTAB		25	408	100	347	80	25
C7H8	toluene	SAS Hypersil Shandon - 5 μ m	CTAB	NaCl -0.1M	25	428	105	365	100	25
C7H8	toluene	SAS Hypersil Shandon - 5 μ m	CTAB	5% MeOH	25	290	71	246	60	25
C7H8	toluene	ODS Hypersil Shandon 5 μ m	CTAB		25	578	142	493	200	25
C7H8	toluene	ODS Hypersil Shandon 5 μ m	CTAB	NaCl -0.1M	25	586	144	500		25
C7H8	toluene	ODS Hypersil Shandon 5 μ m	CTAB	5% MeOH	25	347	85	295	130	25
C7H8	toluene	Altex Octyl Ultrasphere	CTAB		31	489	120	329	270	16
C7H8	toluene	Novapack C18 4 μ m	CTAB		25	390	142	389	175	7
C7H8	toluene	Novapack C18 4 μ m	CTAB	5% BuOH	25	196	71	195	70	9
C7H8	toluene	Rainin Microsorb C18 3 μ m	CTAB		35	347	85	295	128	6
C7H8	toluene	Supelcosil LC-18	DOSS	39% ACN			17	38		14
C7H8	toluene	Supelcosil LC-1 5 μ m	DTAB		25	188		140	60	12
C7H8	toluene	Hypersil ODS C18	MB-14				74	246	82	17
C7H8	toluene	Hypersil ODS C18	SB-12				56	166	76	17
C7H8	toluene	Supelcosil LC-1 5 μ m	SDS		25	217	53	185	68	12

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} OF K _{AS}	ref.
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS		25	310	76	264	144	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	184	45	157	69	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	224	55	190	82	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	223	55	190	90	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	179	44	152	55	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	181	44	153	57	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	159	39	135	52	8
C7H8	toluene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	68	17	57	15	8
C7H8	toluene	μ Bondapack C-18 (Waters)	SDS		25	200	49	170	70	18
C7H8	toluene	Hypersil Shandon - 5 μ m	SDS		amb	230	56	196	1	24
C7H8	toluene	CPS Hypersil Shandon 5 μ m	SDS		amb	170	42	144	23	24
C7H8	toluene	SAS Hypersil Shandon 5 μ m	SDS		amb	240	59	204	19	24
C7H8	toluene	MOS Hypersil Shandon 5 μ m	SDS		amb	290	71	247	150	24
C7H8	toluene	ODS Hypersil Shandon 5 μ m	SDS		amb	240	59	204	140	24
C7H8	toluene	SAS Hypersil Shandon 5 μ m	SDS		25	237	58	201	20	25
C7H8	toluene	ODS Hypersil Shandon- 5 μ m	SDS		25	216	53	184	170	25
C7H8	toluene	Rainin Microsorb 5 μ m octyl	SDS		31	225	55	191	210	16
C7H8	toluene	Rainin Microsorb 5 μ m octyl	SDS	3% PrOH	31	216	53	184	160	16
C7H8	toluene	Rainin Microsorb 3 μ m ODS	SDS		31	574	141	489	650	16

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C7H8	toluene	Novapack C18 4 μ m	SDS		25	214	52	182	130	7
C7H8	toluene	Bakerbond C18	SDS		40	54	13	45		4
C7H8	toluene	Novapack C18 4 μ m	SDS	10% BuOH	25	151	37	128	36	9
C7H8	toluene	Novapack C18 4 μ m	SDS	5% BuOH	25	164	40	139	60	9
C7H8	toluene	Novapack C18 4 μ m	SDS	NaCl 0,1M	25	302	74	257	110	9
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS		35	115	28	97	55	19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS		25	231	56	196		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS		35	219	54	186		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS		45	214	52	182		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	146	36	124		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	136	33	115		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	128	31	108		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	118	29	100		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	114	28	96		19
C7H8	toluene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	100	24	85		19
C7H8	toluene	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	265	65	226	128	6
C7H8	toluene	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	41	10	34	45	5
C7H8	toluene	SAS Hypersil Shandon - 5 μ m	SDS	NaCl -0.1M	25	298	73	253	43	25
C7H8	toluene	SAS Hypersil Shandon - 5 μ m	SDS	5% MeOH	25	21	5,0	17	1,4	25
C7H8	toluene	ODS Hypersil Shandon- 5 μ m	SDS	NaCl -0.1M	25	257	63	219	170	25
C7H8	toluene	ODS Hypersil Shandon- 5 μ m	SDS	5% MeOH	25	204	50	174	130	25
C7H8	toluene	Supelcosil LC-18	THPA	60% ACN			13	26		14
C7H8	toluene	Supelcosil LC-18	Tween 60	60% ACN		250	311	237		14

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C7H8CIN3O4S2	hydrochlorothiazide	Spherisorb ODS-2 - 5 μ m	SDS			45	11	37	2.6	3
C7H8CIN3O4S2	hydrochlorothiazide	Spherisorb ODS-2 - 5 μ m	SDS	phosph. buf.	20	42	10	35	2.5	23
C7H8O	4-methylbenzene-1-ol	μ Bondapak C18	SDS		24	95	24	85	23	10
C7H8O	anisele	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	34	12	33	62	11
C7H8O	anisele	Rainin Microsorb C18 3 μ m	CTAB		35	160	39	135	50	6
C7H8O	anisele	Altex ODS	SDS	3% 2-PrOH	38	27	6.3	22	57	11
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS		35	54	13	45	20	19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS		25	119	29	101		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS		35	112	27	95		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS		45	105	26	89		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	55	13	47		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	53	13	44		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	49	12	41		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	51	12	42		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	48	12	40		19
C7H8O	anisele	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	45	11	38		19
C7H8O	anisele	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	99	24	83	36	6
C7H8O	benzyl alcohol	Waters Radial-PAK C18 - 10 μ m	Brij-35			12	2,6		6,0	15
C7H8O	benzyl alcohol		Brij-35	15% EtOH		10	2,2	7,7	4,0	15
C7H8O	benzyl alcohol	Novapack C18 4 μ m	Brij-35		25	19	19.4	16.2	5.3	7
C7H8O	benzyl alcohol	Gaskro Kogyo - Inertsil ODS C18 5 μ m	Brij-35		25	9.5	9.5	7.6	5.0	20
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB		25	49	17.3	47.5	150.5	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	40	14.2	38.9	10.1	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	33	11.6	31.7	7.8	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	26	9.0	24.7	5.0	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	26	9.2	25.2	5.9	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	22	7.8	21.3	4.4	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	15	5.2	14.2	2.5	8
C7H8O	benzyl alcohol	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	11	3.8	10.3	14.6	11
C7H8O	benzyl alcohol	Novapack C18 4 μ m	CTAB		25	38	13.5	37.0	8.7	7
C7H8O	benzyl alcohol	Novapack C18 4 μ m	CTAB	5% BuOH	25	16	5.4	14.8	3.0	9
C7H8O	benzyl alcohol	Rainin Microsorb C18 3 μ m	CTAB		35	54	13.0	45.1	11.2	6
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS		25	49	11.8	41.1	8.8	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	54	13.0	45.0	5.8	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	34	8.2	28.3	5.1	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	30	7.2	25.1	4.0	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	27	6.5	22.6	2.9	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	23	5.5	19.2	3.2	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	15	3.4	11.9	2.6	8
C7H8O	benzyl alcohol	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	20	4.8	16.6	1.7	8
C7H8O	benzyl alcohol	Altex ODS	SDS	3% 2-PrOH	38	9.2	2.0	7.0	7.9	11
C7H8O	benzyl alcohol	Novapack C18 4 μ m	SDS		25	37	8.8	30.5	6.8	7
C7H8O	benzyl alcohol	Bakerbond C18	SDS		40	10	2.3	8.0		4
C7H8O	benzyl alcohol	Novapack C18 4 μ m	SDS	10% BuOH	25	25	6.0	20.8	2.0	9

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C7H8O	benzyl alcohol	Novapack C18 4 μ m	SDS	5% BuOH	25	164	40,0	138,9	20,0	9
C7H8O	benzyl alcohol	Novapack C18 4 μ m	SDS	NaCl 0,1M	25	50	12,0	41,7	8,0	9
C7H8O	benzyl alcohol	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	34	8,0	27,8	5.3	6
C7H8O	cresol	Supelcosil LC-18	Brij 30	60% ACN		29	13	37		14
C7H8O	cresol	Supelcosil LC-18	DOSS	39% ACN			21	47		14
C7H8O	cresol	Supelcosil LC-18	Tween 60	60% ACN		131	162	124		14
C7H8O	o-cresol	TSK-GPC 2000 SW	SDS		25	84	20	71		2
C7H8O	m-cresol	TSK-GPC 2000 SW	SDS		25	88	21	74		2
C7H8O	p-cresol	TSK-GPC 2000 SW	SDS		25	107	26	91		2
C7H8O	p-cresol	Inertsil ODS C18 5 μ m	Brij-35		25	145	161	130	128	20
C7H8O2	2-methylbenzene-1,4-diol	μ Bondapak C18	SDS		24	21	5.2	18	2.4	10
C7H8O2	4-methylbenzene-1,2-diol	μ Bondapak C18	SDS		24	72	19	65	14	10
C8H7N	phenylacetoneitrile	Novapack C18 4 μ m	Brij-35		25	42	44	37	22	7
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB		25	141	51	140	49	8
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	120	43	119	34	8
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	88	32	87	25	8
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	68	24	67	15	8
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	76	27	75	17	8
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	37	13	36	9.2	8
C8H7N	phenylacetoneitrile	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	27	10	26	5.1	8
C8H7N	phenylacetoneitrile	Novapack C18 4 μ m	CTAB		25	141	51	140	34	7
C8H7N	phenylacetoneitrile	Novapack C18 4 μ m	CTAB	5% BuOH	25	39	14	38	7,0	9

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ϕP_{WS} or K_{AS}	ref.
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS		25	126	31	107	31	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	139	34	118	23	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	87	21	73	18	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	60	14	50	13	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	60	14	50	8.9	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	51	12	43	9.4	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	28	6.6	23	6.7	8
C8H7N	phenylacetonitrile	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	32	7.5	26	3.9	8
C8H7N	phenylacetonitrile	Novapak C18 4 μ m	SDS		25	129	31.6	110	28.6	7
C8H7N	phenylacetonitrile	Novapak C18 4 μ m	SDS	10% BuOH	25	58	14,0	49	4,0	9
C8H7N	phenylacetonitrile	Novapak C18 4 μ m	SDS	5% BuOH	25	39	9,0	31	5,0	9
C8H7N	phenylacetonitrile	Novapak C18 4 μ m	SDS	NaCl 0,1M	25	131	32	111	29,0	9
C8H8Cl3N3O4S2	trichloromethiazide	Spherisorb ODS-2 5 μ m	SDS	Phosph. Buf.	20	80	19	67	4.4	23
C8H8F3N3O3S2	hydroflumethiazide	Spherisorb ODS-2 5 μ m	SDS	Phosph. Buf.	20	50	12	42	3.3	23
C8H8O	acetophenone	Waters Radial-PAK C18 - 10 μ m	Brij-35			22	22	1	29	15
C8H8O	acetophenone		Brij-35	15% EtOH		17	17	1	14	15
C8H8O	acetophenone	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	18	6.2	17	28	11
C8H8O	acetophenone	Altex Octyl Ultrasphere	CTAB		31	61	22	60	37	16
C8H8O	acetophenone	Rainin Microsorb C18 3 μ m	CTAB		35	50	18	49	22	6
C8H8O	acetophenone	Altex ODS	SDS	3% 2-PrOH	38	20	4.7	16	25	11
C8H8O	acetophenone	Rainin Microsorb 5 μ m octyl	SDS		31	82	20	69	55	16
C8H8O	acetophenone	Rainin Microsorb 5 μ m octyl	SDS	3% PrOH	31	50	12	42	23	16
C8H8O	acetophenone	Rainin Microsorb C18 3 μ m	SDS		31	70	17	59	67	16

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C8H8O	acetophenone	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	66	16	56	23	6
C8H8O2	methyl benzoate	Waters Radial-PAK C18 - 10 μ m	Brij-35	15% EtOH		36	37	1	60	15
C8H8O2	methyl benzoate		Brij-35			39	40	1	42	15
C8H8O2	methyl benzoate	Rainin Microsorb C18 3 μ m	CTAB		35	114	41	113	53	6
C8H8O2	methyl benzoate	Rainin Microsorb C18 3 μ m	SDS	2% 1-PrOH	25	127	31	108	50	6
C8H8O3	isovanillin	Apex C18 (Golden) - 5 μ m	CTAB		30	91	33	90	17	26
C8H8O3	isovanillin	Apex C8 (Golden CO) - 5 μ m	CTAB		30	114	41	113	21	26
C8H8O3	isovanillin	Apex C18 (Golden) - 5 μ m	SDS		25	162	40	137	10	26
C8H8O3	isovanillin	Apex C8 (Golden CO) - 5 μ m	SDS		25	168	41	143	12	26
C8H8O3	orthovanillin	Apex C18 (Golden) - 5 μ m	CTAB		30	88	32	87	26	26
C8H8O3	orthovanillin	Apex C8 (Golden CO) - 5 μ m	CTAB		30	122	44	120	25	26
C8H8O3	orthovanillin	Apex C18 (Golden) - 5 μ m	SDS		25	187	46	159	18	26
C8H8O3	orthovanillin	Apex C8 (Golden) - 5 μ m	SDS		25	240	59	204	24	26
C8H8O3	vanillin	Apex C18 (Golden) - 5 μ m	CTAB		30	83	30	82	17	26
C8H8O3	vanillin	Apex C8 (Golden CO) - 5 μ m	CTAB		30	348	126	346	38	26
C8H8O3	vanillin	Apex C18 (Golden) - 5 μ m	SDS		25	154	38	131	10	26
C8H8O3	vanillin	Apex C8 (Golden CO) - 5 μ m	SDS		25	155	38	132	14	26
C8H8O4	vanillic acid	Apex C18 (Golden) - 5 μ m	CTAB		30	162	59	161	17	26
C8H8O4	vanillic acid	Apex C8 (Golden CO) - 5 μ m	CTAB		30	128	46	127	20	26
C8H8O4	vanillic acid	Apex C18 (Golden) - 5 μ m	SDS		25	118	29	100	4	26
C8H8O4	vanillic acid	Apex C8 (Golden CO) - 5 μ m	SDS		25	87	21	74	4	26
C8H9NO	acetanilide	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	17	5.8	16	17	11
C8H9NO	acetanilide	Altex ODS	SDS	3% 2-PrOH	38	13	2.9	10	6.8	11
C8H10	ethylbenzene	Supelcosil LC-18	Brij 30	60% ACN		67	31	87		14

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C8H10	ethylbenzene	Altex Octyl Ultrasphere	CTAB		31	4122	1500	4117	3000	16
C8H10	ethylbenzene	Rainin Microsorb C18 3µm	CTAB		35	421	153	420	263	6
C8H10	ethylbenzene	Supelcosil LC-18	DOSS	39% ACN			20	44		14
C8H10	ethylbenzene	Hypersil ODS C18	SB-12				151	449	105	17
C8H10	ethylbenzene	Rainin Microsorb C18 3µm	SDS		31					16
C8H10	ethylbenzene	Rainin Microsorb C18 3µm	SDS	2% 1-PrOH	25	777	191	663	455	6
C8H10	ethylbenzene	Supelcosil LC-18	THPA	60% ACN			14	28		14
C8H10	ethylbenzene	Supelcosil LC-18	Tween 60	60% ACN		302	377	287		14
C8H10	p-xylene	Hypersil ODS C18	MB-14				113	376	193	17
C8H10	p-xylene	Hypersil ODS C18	SB-12				71	211	122	17
C8H10	p-xylene	µBondapack C-18 (Waters)	SDS		25	570	140	486	220	18
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS		25	733	180	625		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS		35	721	177	614		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS		45	802	197	684		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	304	75	259		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	301	74	257		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	277	68	236		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	246	60	209		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	218	53	185		19
C8H10	p-xylene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	167	41	142		19
C8H10N4O2	caffeine	Hypersil Shandon - 5µm	CTAB		amb	34	12	33	0.3	24
C8H10N4O2	caffeine	CPS Hypersil Shandon - 5µm	CTAB		amb	33	12	32	2.0	24

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C8H10N4O2	caffeine	SAS Hypersil Shandon - 5 μ m	CTAB		amb	39	14	38	3.6	24
C8H10N4O2	caffeine	MOS Hypersil - 5 μ m	CTAB		amb	23	8.0	22	1.8	24
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	CTAB		amb	35	12.4	34	2.3	24
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	CTAB		25	39	14,0	38	4,0	25
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	CTAB	NaCl -0.1M	25	28	10,0	27	4,3	25
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	CTAB	5% MeOH	25	20	7,0	19	3,0	25
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	CTAB		25	35	12,0	33	2,5	25
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	CTAB	NaCl -0.1M	25	34	12,0	33		25
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	CTAB	5% MeOH	25	20	7,0	19	1,4	25
C8H10N4O2	caffeine	Hypersil - 5 μ m	SDS		amb	59	14.3	50	1.0	24
C8H10N4O2	caffeine	CPS Hypersil - 5 μ m	SDS		amb	36	8.6	30	3.5	24
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	SDS		amb	40	9.6	33	12.0	24
C8H10N4O2	caffeine	MOS Hypersil - 5 μ m	SDS		amb	37	8.9	31	9.6	24
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	SDS		amb	38	9.1	32	5.6	24
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	SDS		25	38	9,0	31	14,0	25
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	SDS		25	38	9,0	31	6,0	25
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	50	12,0	42	12,0	25
C8H10N4O2	caffeine	SAS Hypersil - 5 μ m	SDS	5% MeOH	25	19	4,5	16	4,0	25

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	34	8,0	28	8,0	25
C8H10N4O2	caffeine	ODS Hypersil - 5 μ m	SDS	5% MeOH	25	17	4,0	14	4,0	25
C8H10O	2-phenylethanol	Novapak C18 4 μ m	Brij-35		25	18	18.1	15	7.7	7
C8H10O	2-phenylethanol	Inertsil ODS C18 5 μ m	Brij-35		25	13	13.8	11	8.0	20
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB		25	83	30.0	82	26.6	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB	3% n-PrOH	25	65	23.4	64	17.2	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	54	19.2	53	13.4	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	42	15.1	41	8.5	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	47	16.8	46	10.0	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	26	9.0	25	6.1	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	23	8.0	22	3.7	8
C8H10O	2-phenylethanol	Novapak C18 4 μ m	CTAB		25	70	25	69	16.1	7
C8H10O	2-phenylethanol	Novapak C18 4 μ m	CTAB	5% BuOH	25	31	11	30	5,0	9
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS		25	85	21	72	18	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	94	23	79	12	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	61	15	51	10	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	50	12	42	8.0	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	47	11	40	5.5	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	79	19	66	5.9	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	20	4.8	17	4.2	8
C8H10O	2-phenylethanol	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	29	6.8	24	2.7	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C8H10O	2-phenylethanol	Novapack C18 4 μ m	SDS		25	118	28.7	100	22.2	7
C8H10O	2-phenylethanol	Novapack C18 4 μ m	SDS	10% BuOH	25	46	11,0	38	3,0	9
C8H10O	2-phenylethanol	Novapack C18 4 μ m	SDS	5% BuOH	25	25	6,0	21	3,4	9
C8H10O	2-phenylethanol	Novapack C18 4 μ m	SDS	NaCl 0,1M	25	99	24	83	18	9
C8H10O	3,5-dimethylphenol	Novapack C18 4 μ m	Brij-35		25	569	605	504	303	7
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	CTAB	5% n-PrOH	25	622	226	620	228	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	443	161	442	116	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	192	69	190	58	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	67	24	66	22	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	72	26	71	12	8
C8H10O	3,5-dimethylphenol	Novapack C18 4 μ m	CTAB		25	685	249	683	263	7
C8H10O	3,5-dimethylphenol	Novapack C18 4 μ m	CTAB	5% BuOH	25	155	56	154	30	9
C8H10O	3,5-dimethylphenol	μ Bondapak C18	SDS		24	230	60	207	55	10
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS		25	236	58	201	65	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	273	67	232	50	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	164	40	140	37	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	101	25	86	24	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	131	32	111	21	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	102	25	86	20	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	60	14	50	14	8
C8H10O	3,5-dimethylphenol	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	54	13	45	7.1	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C8H10O	3,5-dimethylphenol	Novapack C18 4μm	SDS		25	2000	492	1707	556	7
C8H10O	3,5-dimethylphenol	Novapack C18 4μm	SDS	10% BuOH	25	139	34	118	11	9
C8H10O	3,5-dimethylphenol	Novapack C18 4μm	SDS	5% BuOH	25	103	25	87	14	9
C8H10O	3,5-dimethylphenol	Novapack C18 4μm	SDS	NaCl 0.1M	25	229	56	194	56	9
C8H10O	4-ethylbenzene-1-ol	μBondapak C18	SDS		24	145	64	220	60	10
C8H10O	ethylphenol	Supelcosil LC-18	Brij 30	60% ACN		33	15	43		14
C8H10O	ethylphenol	Supelcosil LC-18	DOSS	39% ACN			23	51		14
C8H10O	ethylphenol	Supelcosil LC-18	Tween 60	60% ACN		157	195	148		14
C8H10O	p-ethylphenol	Inertsil ODS C18 5μm	Brij-35		25	323	361	290	304	20
C8H11NO2	dopamine	Spherisorb Ods-2 C18	Brij35	0% EtOH pH 4.8		5.2	4.5	3.8	14.7	27
C8H11NO2	dopamine	Spherisorb Ods-2 C18	Brij35	10% EtOH pH 4.8		4.0	3.2	2.7	2.1	27
C8H11NO2	dopamine	Spherisorb Ods-2 C18	Brij35	10% EtOH pH 4.8		13.4	13.2	11.2	6.5	27
C8H11NO2	dopamine	Spherisorb Ods-2 C18	Brij35	10% EtOH pH 4.8		1.9	1.0	0.8	2.0	27
C8H11NO3	norepinephrine	Spherisorb Ods-2 C18	Brij35	0% EtOH pH 4.8		2.1	1.2	1.0	2.0	27
C8H11NO3	norepinephrine	Spherisorb Ods-2 C18	Brij35	10% EtOH pH 4.8		2.5	1.6	1.4	0.6	27
C8H11NO3	norepinephrine	Spherisorb Ods-2 C18	Brij35	10% EtOH pH 4.8		6.3	5.6	4.8	0.9	27
C8H11NO3	norepinephrine	Spherisorb Ods-2 C18	Brij35	10% EtOH pH 4.8		1.4	0.4	0.3	0.6	27
C9H6O2	coumarin	Apex C18 (Golden) - 5μm	CTAB		30	103	37	102	30	26
C9H6O2	coumarin	Apex C8 (Golden CO) - 5μm	CTAB		30	191	69	189	39	26
C9H6O2	coumarin	Apex C18 (Golden) - 5μm	SDS		25	241	59	205	42	26
C9H6O2	coumarin	Apex C8 (Golden CO) - 5μm	SDS		25	351	86	299	50	26
C9H10O	propiophenone	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	32	11	31	67	11
C9H10O	propiophenone	Altex Octyl Ultrasphere	CTAB		31	212	52	143	110	16

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C9H10O	propiophenone	Altex ODS	SDS	3% 2-PrOH	38	37	8.8	30	63	11
C9H10O	propiophenone	Rainin Microsorb 5µm octyl	SDS		31	139	34	118	120	16
C9H10O	propiophenone	Rainin Microsorb 5µm octyl	SDS	3% PrOH	31	78	19	66	50	16
C9H10O	propiophenone	Rainin Microsorb C18 3µm	SDS		31	99	24	83	130	16
C9H10O3	ethylvanillin	Apex C18 (Golden) - 5µm	CTAB		30	163	59	161	36	26
C9H10O3	ethylvanillin	Apex C8 (Golden CO) - 5µm	CTAB		30	101	36	100	27	26
C9H10O3	ethylvanillin	Apex C18 (Golden) - 5µm	SDS		25	169	41	143	31	26
C9H10O3	ethylvanillin	Apex C8 (Golden CO) - 5µm	SDS		25	272	67	231	40	26
C9H11NO2	phenylalanine	Nucleosil C18 5µm	SDS	3% 2-PrOH	40	37	9.0	31	26	5
C9H11NO3	tyrosine	Bakerbond C18	SDS		40	112	27	95		4
C9H11NO3	tyrosine	Nucleosil C18 5µm	SDS	3% 2-PrOH	40	65	16	55	16	5
C9H12	propylbenzene	Supelcosil LC-18	Brij 30	60% ACN		50	23	64		14
C9H12	propylbenzene	Supelcosil LC-18	DOSS	39% ACN			22	51		14
C9H12	propylbenzene	Hypersil ODS C18	SB-12				423	1262	333	17
C9H12	propylbenzene	Supelcosil LC-18	THPA	60% ACN			15	30		14
C9H12	propylbenzene	Supelcosil LC-18	Tween 60	60% ACN		339	422	322		14
C9H12O	2,4,5-trimethylbenzene-1-ol	µBondapak C18	SDS		24	514	133	463	142	10
C9H12O	4-n-propylbenzene-1-ol	µBondapak C18	SDS		24	450	117	405	138	10
C9H12O	propylphenol	Supelcosil LC-18	Brij 30	60% ACN		37	17	47		14
C9H12O	propylphenol	Supelcosil LC-18	DOSS	39% ACN			24	54		14
C9H12O	propylphenol	Supelcosil LC-18	Tween 60	60% ACN		183	228	174		14
C9H12O2	2,3,5-trimethylbenzene-1,4-di-ol	µBondapak C18	SDS		24	54	14	48	7.4	10
C9H12O2	3-i-propylbenzene-1,2-diol	µBondapak C18	SDS		24	225	58	202	59	10
C9H13NO3	epinephrine	Spherisorb ODS-2 C18	Brij35	0% EtOH pH 4.8		2.6	1.7	1.4	5.7	27

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	Φ_{WS} or K _{AS}	ref.
C9H13NO3	epinephrine	Spherisorb ODS-2 C18	Brij35	10% EtOH pH 4.8		2.9	2.0	1.7	1.1	27
C9H13NO3	epinephrine	Spherisorb ODS-2 C18	Brij35	10% EtOH pH 4.8		6.9	6.3	5.3	1.7	27
C9H13NO3	epinephrine	Spherisorb ODS-2 C18	Brij35	10% EtOH pH 4.8		1.2	0.2	0.2	1.0	27
C9H14NBr	benzyltrimethyl ammonium bromide	Hypersil - 5 μ m	SDS		amb	1700	418	1451	1400	24
C9H14NBr		CPS Hypersil - 5 μ m	SDS		amb	500	123	426	300	24
C9H14NBr	benzyltrimethyl ammonium bromide	SAS Hypersil - 5 μ m	SDS		amb	1700	418	1451	510	24
C9H14NBr		MOS Hypersil - 5 μ m	SDS		amb	2000	492	1707	1600	24
C9H14NBr	benzyltrimethyl ammonium bromide	ODS Hypersil - 5 μ m	SDS		amb	1900	467	1622	1667	24
C9H14NBr		SAS Hypersil - 5 μ m	SDS		25	1708	420	1458	500	25
C9H14NBr	benzyltrimethyl ammonium bromide	ODS Hypersil - 5 μ m	SDS		25	1912	470	1632	1700	25
C9H14NBr		SAS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	570	140	486	140	25
C9H14NBr	benzyltrimethyl ammonium bromide	SAS Hypersil - 5 μ m	SDS	5% MeOH	25	814	200	694	80	25
C9H14NBr		ODS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	529	130	451	900	25
C9H14NBr	benzyltrimethyl ammonium bromide	ODS Hypersil - 5 μ m	SDS	5% MeOH	25	936	230	798	2000	25
C10H7Br	1-bromonaphthalene	μ Bondapack C-18	SDS		25	18700	4600	15969	7700	18
C10H8	naphthalene	Supelcosil LC-18	Brij 30	60% ACN		49	23	63		14
C10H8	naphthalene	Novapak C18 - 4 μ m	Brij-35		25	40	41	35	75	30
C10H8	naphthalene	Hypersil C18 5 μ m	Brij-35			39	40	34	40	28
C10H8	naphthalene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	706	257	704	242	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C10H8	naphthalene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	193	70	192	70	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	110	40	109	27	8
C10H8	naphthalene	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	180	65	179	417	11
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	5% BuOH	25	998	363	996	330	9
C10H8	naphthalene	Novapak C18 - 4 μ m	CTAB		40	114	41	113	40	30
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	5% MeOH	40	1139	414	1136		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	10% MeOH	40	1096	398	1093		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	15% MeOH	40	980	356	977		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	825	300	822		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	731	266	729		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	368	134	366		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	711	258	709		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	382	139	381		29
C10H8	naphthalene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	310	112	309		29
C10H8	naphthalene	Supelcosil LC-18	DOSS	39% ACN			23	51		14
C10H8	naphthalene	Micropak CN 10 μ m	SDS		22	317	78	272	5.2	13
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS		25	884	217	754	519	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	1895	466	1617	780	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	923	227	787	390	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	843	207	719	383	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	480	118	409	161	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ϕP_{WS} or K_{AS}	ref.
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	395	97	336	142	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	99	24	83	48	8
C10H8	naphthalene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	87	21	74	20	8
C10H8	naphthalene	Altex ODS	SDS	3% 2-PrOH	38	238	58	202	909	11
C10H8	naphthalene	μ Bondapack C-18 (Waters)	SDS		25	956	235	816	320	18
C10H8	naphthalene	Novapack C18 4 μ m	SDS		25	1216	299	1038	833	7
C10H8	naphthalene	Bakerbond C18	SDS		40	235	58	200		4
C10H8	naphthalene	Novapack C18 4 μ m	SDS	10% BuOH	25	245	60	208	70	9
C10H8	naphthalene	Novapack C18 4 μ m	SDS	5% BuOH	25	286	70	243	130	9
C10H8	naphthalene	Novapack C18 4 μ m	SDS	NaCl 0,1M	25	481	118	410	230	9
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS		35	204	50	174	100	19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS		25	1436	353	1225		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS		35	1473	362	1257		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS		45	1009	248	861		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	210	51	178		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	208	51	177		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	192	47	163		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	171	42	145		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	168	41	142		19
C10H8	naphthalene	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	163	40	139		19
C10H8	naphthalene	Novapak C18 - 4 μ m	SDS		25	1001	246	854	730	30
C10H8	naphthalene	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	104	25	89	138	5
C10H8	naphthalene	Novapack C18 4 μ m	SDS	5% MeOH	25	324	79	275		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ΦP_{WS} or K_{AS}	ref.
C10H8	naphthalene	Novapack C18 4 μ m	SDS	10% MeOH	25	301	74	256		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	15% MeOH	25	271	66	230		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	20% MeOH	25	249	61	212		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	82	20	69		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	129	32	110		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	164	40	139		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	297	73	253		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	272	67	231		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	212	52	180		29
C10H8	naphthalene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	208	51	177		29
C10H8	naphthalene	Supelcosil LC-18	THPA	60% ACN			15	31		14
C10H8	naphthalene	Supelcosil LC-18	Tween 60	60% ACN		205	256	195		14
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	2143	780	2140	561	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	331	120	329	105	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	92	33	91	30	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	171	62	170	26	8
C10H8O	1-naphthol	Novapack C18 4 μ m	CTAB	5% BuOH	25	336	122	335	60	9
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS		25	771	190	658	254	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	505	124	430	117	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	325	80	277	80	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	212	52	180	52	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	10% n-PrOH	25	253	62	216	44	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	183	45	155	38	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	104	25	88	25	8
C10H8O	1-naphthol	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	73	18	61	10	8
C10H8O	1-naphthol	Novapack C18 4 μ m	SDS		25	432	106	368	130	7
C10H8O	1-naphthol	Novapack C18 4 μ m	SDS	10% BuOH	25	229	56	194	20	9
C10H8O	1-naphthol	Novapack C18 4 μ m	SDS	5% BuOH	25	160	39	135	26	9
C10H8O	1-naphthol	Novapack C18 4 μ m	SDS	NaCl 0.1M	25	814	200	694	200	9
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	CTAB	10% n-PrOH	25	1643	598	1641	416	8
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	288	105	287	92	8
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	85	31	84	28	8
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	126	45	124	19	8
C10H8O	2-naphthol	Novapack C18 4 μ m	CTAB	5% BuOH	25	325	118	324	60	9
C10H8O	2-naphthol	Supelcosil LC-1 5 μ m	DTAB		25	2	135	1	210	12
C10H8O	2-naphthol	Supelcosil LC-1 5 μ m	SDS		25	2	100	1	100	12
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	SDS		25	684	168	583	226	8
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	SDS	10% MeOH	25	596	146	508	131	8
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	299	73	254	71	8
C10H8O	2-naphthol	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	248	61	211	57	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C10H8O	2-naphthol	Spherisorb C8 - 5μm	SDS	10% n-PrOH	25	226	55	192	37	8
C10H8O	2-naphthol	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	171	42	145	34	8
C10H8O	2-naphthol	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	120	29	102	25	8
C10H8O	2-naphthol	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	67	16	57	9	8
C10H8O	2-naphthol	Novapack C18 4μm	SDS		25	1081	266	921	294	7
C10H8O	2-naphthol	Novapack C18 4μm	SDS	10% BuOH	25	204	50	174	17	9
C10H8O	2-naphthol	Novapack C18 4μm	SDS	5% BuOH	25	143	35	122	21	9
C10H8O	2-naphthol	Novapack C18 4μm	SDS	NaCl 0,1M	25	977	240	833	242	9
C10H9N	1-naphthylamine	Novapack C18 4μm	CTAB		25	878	319	877	256	7
C10H9N	1-naphthylamine	Novapack C18 4μm	CTAB	5% BuOH	25	199	72	198	33	9
C10H9N	1-naphthylamine	Novapack C18 4μm	SDS	10% BuOH	25	90	22	76	9	9
C10H9N	1-naphthylamine	Novapack C18 4μm	SDS	5% BuOH	25	86	21	73	15	9
C10H9N	1-naphthylamine	Novapack C18 4μm	SDS	NaCl 0,1M	25	672	165	573	188	9
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	CTAB	5% n-PrOH	25	1823	663	1820	544	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	CTAB	10% n-PrOH	25	537	195	535	122	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	CTAB	3% n-BuOH	25	244	89	243	65	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	CTAB	5% n-BuOH	25	103	37	102	27	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	73	26	72	12	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS		25	1009	248	861	315	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS	3% n-PrOH	25	254	62	216	64	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS	5% n-PrOH	25	182	45	155	45	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS	10% n-PrOH	25	144	35	122	25	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	131	32	111	28	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	76	18	64	17	8
C10H9N	1-naphthylamine	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	53	13	45	7.0	8
C10H9NO2	indole 3-yl acetic acid	R-CN Lichrospher 5μm	SDS			66	16	56	3.6	29
C10H10O4	dimethyl terephthalate	Radial-PAK C18 - 10μm	Brij-35			82	86	73	154	15
C10H10O4	dimethyl terephthalate	Radial-PAK C18 - 10μm	Brij-35	15% EtOH		57	60	50	65	15
C10H13O	butyrophenone	Altex C8 Ultrasphere	CTAB	3% 2-PrOH	38	61	22	60	161	11
C10H13O	butyrophenone	Altex Octyl Ultrasphere	CTAB		31	366	133	365	350	16
C10H13O	butyrophenone	Altex ODS	SDS	3% 2-PrOH	38	63	15	53	147	11
C10H13O	butyrophenone	Rainin Microsorb 5μm octyl	SDS		31	233	57	198	270	16
C10H13O	butyrophenone	Rainin Microsorb 5μm octyl	SDS	3% PrOH	31	115	28	97	90	16
C10H13O	butyrophenone	Microsorb 3μm - ODS	SDS		31	155	38	132	270	16
C10H14	butylbenzene	Supelcosil LC-18	Brij 30	60% ACN		55	26	71		14
C10H14	butylbenzene	Supelcosil LC-18	DOSS	39% ACN			27	61		14
C10H14	butylbenzene	Hypersil ODS C18	SB-12				36	107	34	17
C10H14	butylbenzene	Supelcosil LC-18	THPA	60% ACN			17	35		14
C10H14	butylbenzene	Supelcosil LC-18	Tween 60	60% ACN		391	488	372		14
C10H14O	2,3,5,6-tetramethylbenzene-1-ol	μBondapak C18	SDS		24	1060	275	956	359	10
C10H14O	4-t-butylbenzene-1-ol	μBondapak C18	SDS		24	995	258	897	307	10
C10H14O	butylphenol	Supelcosil LC-18	Brij 30	60% ACN		42	20	54		14
C10H14O	butylphenol	Supelcosil LC-18	DOSS	39% ACN			27	60		14

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	φP _{WS} or K _{AS}	ref.
C10H14O	butylphenol	Supelcosil LC-18	Tween 60	60% ACN		218	271	207		14
C10H14O	p-tert-butylphenol	Inertsil ODS C18 5μm	Brij-35		25	1100	1231	989	1482	20
C10H14O2	2-t-butylbenzene-1,4-diol	μBondapak C18	SDS		24	244	63	220	40	10
C10H14O2	4-t-butylbenzene-1,2-diol	μBondapak C18	SDS		24	431	112	388	123	10
C11H10	1-methylnaphthalene	μBondapak C-18	SDS		25	4879	1200	4166	1900	18
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS		25	306	75	260		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS		35	284	70	242		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS		45	261	64	222		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS	3% PrOH	25	82	20	69		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS	3% PrOH	35	79	19	67		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS	3% PrOH	45	76	18	64		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS	10% PrOH	25	80	20	68		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS	10% PrOH	35	74	18	62		19
C11H10O	1-naphthalenemethanol	Brownlee RP-18 Spheri-10	SDS	10% PrOH	45	70	17	59		19
C11H11NO2	indole 3-yl propanoic acid	R-CN Lichrospher 5μm	SDS			251	61	213	11	29
C11H12N2O2	tryptophan	Bakerbond C18	SDS		40	421	103	359		4
C11H12N2O2	tryptophan	Nucleosil C18 5μm	SDS	3% 2-PrOH	40	154	38	131	96	5
C11H14ClN3O4S3	althiazide	Spherisorb ODS-2 5μm	SDS	phosph. buf.	20	260	64	221	36	23
C11H14O	valerophenone	Altex Octyl Ultrasphere	CTAB		31	1737	427	1172	1400	16
C11H14O	valerophenone	Rainin Microsorb 5μm octyl	SDS		31	399	98	340	620	16
C11H14O	valerophenone	Rainin Microsorb 5μm octyl	SDS	3% PrOH	31	147	36	125	140	16
C11H14O	valerophenone	Rainin Microsorb 3μm ODS	SDS		31	375	92	319	830	16
C11H16O	pentylphenol	Supelcosil LC-18	Brij 30	60% ACN		45	21	58		14

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C11H16O	pentylphenol	Supelcosil LC-18	DOSS	39% ACN			31	69		14
C11H16O	pentylphenol	Supelcosil LC-18	Tween 60	60% ACN		254	317	241		14
C11H17NO3	isoproterenol	Spherisorb ODS-2 C18	Brij35	0% EtOH pH 4.8		7.1	6.5	5.5	28.4	27
C11H17NO3	isoproterenol	Spherisorb ODS-2 C18	Brij35	10% EtOH pH 4.8		5.0	4.3	3.6	3.1	27
C11H17NO3	isoproterenol	Spherisorb ODS-2 C18	Brij35	10% EtOH pH 4.8		10.5	10.1	8.6	5.1	27
C11H17NO3	isoproterenol	Spherisorb ODS-2 C18	Brij35	10% EtOH pH 4.8		3.1	2.2	1.9	2.9	27
C12H8	acenaphthylene	Novapak C18 - 4 μ m	Brij-35		25	39	40	34	70	30
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	1059	385	1057	371	8
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	218	79	217	80	8
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	130	47	129	32	8
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	5% MeOH	40	1584	576	1581		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	10% MeOH	40	1484	540	1481		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	15% MeOH	40	1316	479	1313		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	1355	493	1352		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	948	345	945		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	496	180	494		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	878	319	876		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	598	217	596		29
C12H8	acenaphthylene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	431	157	430		29
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	2018	496	1722	946	8
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	797	196	680	406	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	527	129	449	205	8
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	230	56	196	100	8
C12H8	acenaphthylene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	91	22	77	22	8
C12H8	acenaphthylene	Novapak C18 - 4 μ m	SDS		25	1708	420	1458	1300	30
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	5% MeOH	25	560	138	477		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	10% MeOH	25	504	124	429		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	15% MeOH	25	369	91	314		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	20% MeOH	25	320	78	272		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	10% 1-BuOH	25	93	23	78		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	7% 1-BuOH	25	180	44	153		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	5% 1-BuOH	25	192	47	163		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	5% 2-PrOH	25	526	129	448		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	10% 2-PrOH	25	470	115	400		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	15% 2-PrOH	25	337	83	287		29
C12H8	acenaphthylene	Novapak C18 4 μ m	SDS	20% 2-PrOH	25	313	77	266		29
C12H10	acenaphthene	Hypersil C18 5 μ m	Brij-35			95	100	85	109	28
C12H10	acenaphthene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	2277	828	2274	886	8
C12H10	acenaphthene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	164	59	162	72	8
C12H10	acenaphthene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	120	43	119	33	8
C12H10	acenaphthene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	1549	381	1322	811	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{WS} or K _{AS}	ref.
C12H10	acenaphthene	Spherisorb C8 - 5μm	SDS	5% n-PrOH	25	774	190	660	462	8
C12H10	acenaphthene	Spherisorb C8 - 5μm	SDS	3% n-BuOH	25	1014	249	865	423	8
C12H10	acenaphthene	Spherisorb C8 - 5μm	SDS	5% n-BuOH	25	291	71	248	133	8
C12H10	acenaphthene	Spherisorb C8 - 5μm	SDS	10% n-BuOH	25	95	23	80	24	8
C12H10	biphenyl	μBondapak C-18 (Waters)	SDS		25	5692	1400	4860	2200	18
C12H10O2	1-naphthylacetic acid	R-CN Lichrospher 5μm	SDS			167	41	142	7.3	21
C12H10O2	2-(2-naphthyl)acetic acid	R-CN Lichrospher 5μm	SDS			217	53	185	10	21
C12H11CIN2O5S	furosemide	Spherisorb ODS-2 - 5μm	SDS			25	5.8	20	3.9	3
C12H11NO	2-(1-naphthyl) acetamide	R-CN Lichrospher 5μm	SDS			313	77	266	14	21
C12H11O2	2-phenylbenzene-1,4-diol	μBondapak C18	SDS		24	202	52	181	26	10
C12H13NO2	ethyl indole 3-yl acetate	R-CN Lichrospher 5μm	SDS			72	17	60	29	21
C12H13NO2	indole 3-yl butyric acid	R-CN Lichrospher 5μm	SDS			334	82	284	14	21
C12H16N2O4	alanyltyrosine	Nucleosil C18 5μm	SDS	3% 2-PrOH	40	109	26	93	15	5
C12H16N2O4	alanyltyrosine	Bakerbond C18	SDS		40	318	78	271		4
C13H10	fluorene	Novapak C18 - 4μm	Brij-35		25	49	51	43	110	30
C13H10	fluorene	Spherisorb C8 - 5μm	CTAB	3% n-BuOH	25	2304	838	2300	884	8
C13H10	fluorene	Spherisorb C8 - 5μm	CTAB	5% n-BuOH	25	272	99	271	107	8
C13H10	fluorene	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	134	48	132	35	8
C13H10	fluorene	Novapak C18 4μm	CTAB	5% MeOH	40	2065	751	2061		29
C13H10	fluorene	Novapak C18 4μm	CTAB	10% MeOH	40	1680	611	1677		29
C13H10	fluorene	Novapak C18 4μm	CTAB	15% MeOH	40	1499	545	1495		29
C13H10	fluorene	Novapak C18 4μm	CTAB	3% 2-PrOH	40	1650	600	1646		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{ws} or K _{AS}	ref.
C13H10	fluorene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	1062	386	1060		29
C13H10	fluorene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	783	285	781		29
C13H10	fluorene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	1003	365	1001		29
C13H10	fluorene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	870	316	868		29
C13H10	fluorene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	712	259	710		29
C13H10	fluorene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	5393	1326	4605	2962	8
C13H10	fluorene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	2154	530	1839	1253	8
C13H10	fluorene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	730	179	622	318	8
C13H10	fluorene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	252	62	214	122	8
C13H10	fluorene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	92	22	78	23	8
C13H10	fluorene	Novapak C18 - 4 μ m	SDS		25	10977	2700	9373	11000	30
C13H10	fluorene	Novapack C18 4 μ m	SDS	5% MeOH	25	1730	425	1476		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	10% MeOH	25	1453	357	1239		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	15% MeOH	25	936	230	798		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	20% MeOH	25	520	128	443		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	110	27	93		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	240	59	204		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	244	60	207		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	1220	300	1040		29
C13H10	fluorene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	846	208	721		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C14H10	anthracene	Novapack C18 4 μ m	CTAB	10% MeOH	40	2025	737	2021		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	15% MeOH	40	1570	571	1567		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	1983	721	1979		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	1647	599	1644		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	1044	380	1042		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	1337	486	1334		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	1191	433	1188		29
C14H10	anthracene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	898	326	895		29
C14H10	anthracene	Supelcosil LC-18	DOSS	39% ACN			33	75		14
C14H10	anthracene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	1346	331	1149	858	8
C14H10	anthracene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	933	229	796	429	8
C14H10	anthracene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	374	92	319	178	8
C14H10	anthracene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	107	26	90	27	8
C14H10	anthracene	Altex ODS	SDS	3% 2-PrOH	38	409	100	348	2500	11
C14H10	anthracene	μ Bondapack C-18	SDS		25	20733	5100	17705	9000	18
C14H10	anthracene	Bakerbond C18	SDS		40	15000	3690	12809		4
C14H10	anthracene	Novapak C18 - 4 μ m	SDS		25	21952	5400	18746	26000	30
C14H10	anthracene	nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	279	68	240	582	5
C14H10	anthracene	Novapack C18 4 μ m	SDS	5% MeOH	25	2053	505	1751		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	10% MeOH	25	1808	444	1541		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	15% MeOH	25	1143	281	974		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	20% MeOH	25	991	244	845		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C14H10	anthracene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	129	31	109		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	286	70	243		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	292	72	248		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	1484	365	1265		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	1245	306	1062		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	789	194	672		29
C14H10	anthracene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	555	136	472		29
C14H10	anthracene	Supelcosil LC-18	THPA	60% ACN			21	42		14
C14H10	anthracene	Supelcosil LC-18	Tween 60	60% ACN		195	243	185		14
C14H10	phenanthrene	Novapak C18 - 4 μ m	Brij-35		25	49	51	43	100	30
C14H10	phenanthrene	Hypersil C18 5 μ m	Brij-35			88	93	79	86	28
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	1824	664	1821	667	8
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	286	104	285	110	8
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	165	60	164	41	8
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	5% MeOH	40	2805	1020	2800		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	10% MeOH	40	2068	752	2064		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	15% MeOH	40	1787	650	1784		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	1924	700	1920		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	1618	588	1615		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	991	360	989		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	1193	434	1190		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	1118	407	1116		29
C14H10	phenanthrene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	896	326	894		29
C14H10	phenanthrene	nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	188	46	161	306	5
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	SDS	3% n-PrOH	25	258	63	220	204	8
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	658	162	561	429	8
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	708	174	604	323	8
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	307	75	261	147	8
C14H10	phenanthrene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	91	22	77	24	8
C14H10	phenanthrene	Bakerbond C18	SDS		40	1220	300	1041		4
C14H10	phenanthrene	Novapak C18 - 4 μ m	SDS		25	15042	3700	12845	16000	30
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	5% MeOH	25	1983	488	1691		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	10% MeOH	25	1540	379	1313		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	15% MeOH	25	1003	246	855		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	20% MeOH	25	905	222	771		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	125	31	106		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	251	62	214		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	256	63	217		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	1450	356	1236		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	1066	262	909		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ϕP_{WS} or K_{AS}	ref.
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	772	190	658		29
C14H10	phenanthrene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	583	143	496		29
C14H11CIN2O4S	chlorthalidone	Spherisorb ODS-2 - 5 μ m	SDS			412	101	351	56	3
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	5% MeOH	40	12574	4577	12556		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	10% MeOH	40	3044	1108	3039		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	15% MeOH	40	2609	949	2604		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	4140	1507	4133		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	2435	886	2430		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	1324	482	1321		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	1375	500	1372		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	1277	464	1274		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	1181	430	1178		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	5% MeOH	25	2540	625	2166		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	10% MeOH	25	2344	576	1999		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	15% MeOH	25	1413	347	1205		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	20% MeOH	25	1288	317	1098		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	95	23	80		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	360	88	306		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	370	91	315		29
C14H12	9-methylanthracene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	2224	547	1897		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C14H12	9-methylanthracene	Novapak C18 4 μ m	SDS	10% 2-PrOH	25	1448	356	1234		29
C14H12	9-methylanthracene	Novapak C18 4 μ m	SDS	15% 2-PrOH	25	929	228	792		29
C14H12	9-methylanthracene	Novapak C18 4 μ m	SDS	20% 2-PrOH	25	767	188	654		29
C14H12	9-methyl-anthracene	Novapak C18 - 4 μ m	Brij-35		25	123	130	110	275	30
C14H12	9-methyl-anthracene	Novapak C18 - 4 μ m	SDS		25	36993	9100	31591	50000	30
C14H16N2O4S	dansyl-glycine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	38	9.2	32	11	5
C14H21NaO3S	sodium octylbenzenesulfonate	Hypersil - 5 μ m	SDS		amb	160	39	136	0.2	24
C14H21NaO3S		CPS Hypersil - 5 μ m	SDS		amb	110	27	93	10	24
C14H21NaO3S	sodium octylbenzenesulfonate	SAS Hypersil - 5 μ m	SDS		amb	160	39	136	9.4	24
C14H21NaO3S		MOS Hypersil - 5 μ m	SDS		amb	300	74	255	35	24
C14H21NaO3S	sodium octylbenzenesulfonate	ODS Hypersil - 5 μ m	SDS		amb	220	54	187	33	24
C14H21NaO3S		SAS Hypersil - 5 μ m	SDS		25	155	38	132	11	25
C14H21NaO3S	sodium octylbenzenesulfonate	ODS Hypersil - 5 μ m	SDS		25	221	54	187	36	25
C14H21NaO3S		SAS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	651	160	555	83	25
C14H21NaO3S	sodium octylbenzenesulfonate	SAS Hypersil - 5 μ m	SDS	5% MeOH	25	139	34	118	8,0	25
C14H21NaO3S		ODS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	408	100	347	48	25
C14H21NaO3S	sodium octylbenzenesulfonate	ODS Hypersil - 5 μ m	SDS	5% MeOH	25	155	38	132	22	25

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP _{ws} or K _{AS}	ref.
C14H21NO4	dihydropyridine ester 24	Spherisorb ODS-2 - 5μm	CTAB	% n-PrOH	30	171	62	170	64	31
C14H21NO4		Spherisorb ODS-2 - 5μm	SDS	% n-PrOH	30	375	92	319	81	31
C15H14F3N3O4S2	bendroflumethiazide	Spherisorb ODS-2 - 5μm	SDS			310	76	264	62	23
C15H22N2O4	leucine-tyrosine	Nucleosil C18 5μm	SDS	3% 2-PrOH	40	138	34	118	45	5
C15H22N2O4	leucyl-tyrosine	Bakerbond C18	SDS		40	553	136	471		4
C16H10	fluoranthene	Novapak C18 - 4μm	Brij-35		25	43	45	38	90	30
C16H10	fluoranthene	Hypersil C18 5μm	Brij-35			368	390	331	339	28
C16H10	fluoranthene	Spherisorb C8 - 5μm	CTAB	3% n-BuOH	25	2666	970	2662	1052	8
C16H10	fluoranthene	Spherisorb C8 - 5μm	CTAB	5% n-BuOH	25	271	98	270	108	8
C16H10	fluoranthene	Spherisorb C8 - 5μm	CTAB	10% n-BuOH	25	197	71	196	49	8
C16H10	fluoranthene	Novapack C18 4μm	CTAB	5% MeOH	40	2944	1071	2939		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	10% MeOH	40	2266	825	2262		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	15% MeOH	40	1992	725	1988		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	3% 2-PrOH	40	2050	746	2046		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	5% 2-PrOH	40	1671	608	1668		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	10% 2-PrOH	40	1106	402	1104		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	5% 1-BuOH	40	1329	483	1326		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	7% 1-BuOH	40	1217	443	1215		29
C16H10	fluoranthene	Novapack C18 4μm	CTAB	10% 1-BuOH	40	999	363	996		29
C16H10	fluoranthene	Spherisorb C8 - 5μm	SDS	5% n-PrOH	25	1531	376	1307	1054	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	933	229	796	462	8
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	318	78	271	164	8
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	103	25	87	27	8
C16H10	fluoranthene	Novapak C18 - 4 μ m	SDS		25	30489	7500	26037	38000	30
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	5% MeOH	25	1722	423	1469		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	10% MeOH	25	1526	375	1301		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	15% MeOH	25	1415	348	1207		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	20% MeOH	25	1345	331	1147		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	10% 1-BuOH	25	132	32	112		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	7% 1-BuOH	25	211	52	179		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	5% 1-BuOH	25	428	105	364		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	5% 2-PrOH	25	1662	409	1418		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	10% 2-PrOH	25	1222	300	1042		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	15% 2-PrOH	25	759	186	647		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	20% 2-PrOH	25	688	169	586		29
C16H10	pyrene	Supelcosil LC-18	Brij 30	60% ACN		62	29	81		14
C16H10	pyrene	Novapak C18 - 4 μ m	Brij-35		25	52	54	46	107	30
C16H10	pyrene	Hypersil C18 - 5 μ m	Brij-35			302	320	272	276	28
C16H10	pyrene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	3226	1174	3222	1291	8
C16H10	pyrene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	271	98	270	109	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{ws} or K _{AS}	ref.
C16H10	pyrene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	186	67	185	47	8
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB		40	633	230	631	360	30
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	5% MeOH	40	13053	4751	13034		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	10% MeOH	40	3258	1186	3253		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	15% MeOH	40	2433	885	2429		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	3% 2-PrOH	40	4336	1578	4329		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	5% 2-PrOH	40	2496	908	2491		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	10% 2-PrOH	40	1674	609	1671		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	5% 1-BuOH	40	2042	743	2038		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	7% 1-BuOH	40	1485	540	1482		29
C16H10	pyrene	Novapak C18 - 4 μ m	CTAB	10% 1-BuOH	40	1203	438	1201		29
C16H10	pyrene	Supelcosil LC-18	DOSS	39% ACN			36	82		14
C16H10	pyrene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	1686	415	1439	1164	8
C16H10	pyrene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	801	197	683	400	8
C16H10	pyrene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	276	68	235	143	8
C16H10	pyrene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	94	23	80	25	8
C16H10	pyrene	μ Bondapak C-18	SDS		25	35773	8800	30550	17000	18
C16H10	pyrene	Novapak C18 - 4 μ m	SDS		25	34147	8400	29161	44000	30
C16H10	pyrene	nucleosil C18 - 5 μ m	SDS	3% 2-PrOH	40	361	89	310	864	5
C16H10	pyrene	Novapak C18 - 4 μ m	SDS	5% MeOH	25	2619	644	2234		29
C16H10	pyrene	Novapak C18 - 4 μ m	SDS	10% MeOH	25	2226	547	1899		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	15% MeOH	25	1918	472	1636		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	20% MeOH	25	1582	389	1349		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	10% 1-BuOH	25	174	42	147		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	7% 1-BuOH	25	376	92	320		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	5% 1-BuOH	25	399	98	339		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	5% 2-PrOH	25	2447	602	2087		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	10% 2-PrOH	25	1686	414	1438		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	15% 2-PrOH	25	944	232	805		29
C16H10	pyrene	Novapack C18 - 4 μ m	SDS	20% 2-PrOH	25	735	180	626		29
C16H10	pyrene	Supelcosil LC-18	Tween 60	60% ACN		234	292	222		14
C17H12	2,3-benzofluorene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	276	100	275	118	8
C17H12	2,3-benzofluorene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	192	70	191	49	8
C17H12	2,3-benzofluorene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	1863	458	1590	1008	8
C17H12	2,3-benzofluorene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	380	93	324	213	8
C17H12	2,3-benzofluorene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	108	26	92	29	8
C17H22N2O4S2	dansyl-methionine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	45	11	38	25	5
C17H23N3O3	leucine-tryptophan	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	193	47	166	162	5
C17H23N3O3	leucyl-tryptophan	Bakerbond C18	SDS		40	1410	347	1203		4
C17H25N3O5	glycyl-leucyl-tyrosine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	189	46	162	59	5

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C17H25N3O5	glycyl-leucyl-tyrosine	Bakerbond C18	SDS		40	401	98	342		4
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	Brij-35		25	198	210	178	430	30
C18H12	benzo(a)anthracene	Hypersil C18 5 μ m	Brij-35			311	330	280	322	28
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	5% MeOH	40	28847	10500	28817		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	10% MeOH	40	12364	4500	12350		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	15% MeOH	40	7144	2600	7136		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	12364	4500	12350		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	9616	3500	9606		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	5770	2100	5763		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	4946	1800	4940		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	4397	1600	4391		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	3160	1150	3156		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS		25	73578	18100	62835	127000	30
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	5% MeOH	25	9313	2291	7946		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	10% MeOH	25	9053	2227	7724		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	15% MeOH	25	7805	1920	6659		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	20% MeOH	25	7568	1862	6457		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	227	56	193		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	703	173	599		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	917	225	781		29
C18H12	benzo(a)anthracene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	6515	1602	5558		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C14H12	9-methylanthracene	Novapak C18 4 μ m	SDS	10% 2-PrOH	25	1448	356	1234		29
C14H12	9-methylanthracene	Novapak C18 4 μ m	SDS	15% 2-PrOH	25	929	228	792		29
C14H12	9-methylanthracene	Novapak C18 4 μ m	SDS	20% 2-PrOH	25	767	188	654		29
C14H12	9-methyl-anthracene	Novapak C18 - 4 μ m	Brij-35		25	123	130	110	275	30
C14H12	9-methyl-anthracene	Novapak C18 - 4 μ m	SDS		25	36993	9100	31591	50000	30
C14H16N2O4S	dansyl-glycine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	38	9.2	32	11	5
C14H21NaO3S	sodium octylbenzenesulfonate	Hypersil - 5 μ m	SDS		amb	160	39	136	0.2	24
C14H21NaO3S		CPS Hypersil - 5 μ m	SDS		amb	110	27	93	10	24
C14H21NaO3S	sodium octylbenzenesulfonate	SAS Hypersil - 5 μ m	SDS		amb	160	39	136	9.4	24
C14H21NaO3S		MOS Hypersil - 5 μ m	SDS		amb	300	74	255	35	24
C14H21NaO3S	sodium octylbenzenesulfonate	ODS Hypersil - 5 μ m	SDS		amb	220	54	187	33	24
C14H21NaO3S		SAS Hypersil - 5 μ m	SDS		25	155	38	132	11	25
C14H21NaO3S	sodium octylbenzenesulfonate	ODS Hypersil - 5 μ m	SDS		25	221	54	187	36	25
C14H21NaO3S		SAS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	651	160	555	83	25
C14H21NaO3S	sodium octylbenzenesulfonate	SAS Hypersil - 5 μ m	SDS	5% MeOH	25	139	34	118	8,0	25
C14H21NaO3S		ODS Hypersil - 5 μ m	SDS	NaCl -0.1M	25	408	100	347	48	25
C14H21NaO3S	sodium octylbenzenesulfonate	ODS Hypersil - 5 μ m	SDS	5% MeOH	25	155	38	132	22	25

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{ws} or K _{AS}	ref.
C14H21NO4	dihydropyridine ester 24	Spherisorb ODS-2 - 5 μ m	CTAB	% n-PrOH	30	171	62	170	64	31
C14H21NO4		Spherisorb ODS-2 - 5 μ m	SDS	% n-PrOH	30	375	92	319	81	31
C15H14F3N3O4S2	bendroflumethiazide	Spherisorb ODS-2 - 5 μ m	SDS			310	76	264	62	23
C15H22N2O4	leucine-tyrosine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	138	34	118	45	5
C15H22N2O4	leucyl-tyrosine	Bakerbond C18	SDS		40	553	136	471		4
C16H10	fluoranthene	Novapak C18 - 4 μ m	Brij-35		25	43	45	38	90	30
C16H10	fluoranthene	Hypersil C18 5 μ m	Brij-35			368	390	331	339	28
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	2666	970	2662	1052	8
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	271	98	270	108	8
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	CTAB	10% n-BuOH	25	197	71	196	49	8
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	5% MeOH	40	2944	1071	2939		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	10% MeOH	40	2266	825	2262		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	15% MeOH	40	1992	725	1988		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	2050	746	2046		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	1671	608	1668		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	1106	402	1104		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	1329	483	1326		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	1217	443	1215		29
C16H10	fluoranthene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	999	363	996		29
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	5% n-PrOH	25	1531	376	1307	1054	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	3% n-BuOH	25	933	229	796	462	8
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	5% n-BuOH	25	318	78	271	164	8
C16H10	fluoranthene	Spherisorb C8 - 5 μ m	SDS	10% n-BuOH	25	103	25	87	27	8
C16H10	fluoranthene	Novapak C18 - 4 μ m	SDS		25	30489	7500	26037	38000	30
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	5% MeOH	25	1722	423	1469		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	10% MeOH	25	1526	375	1301		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	15% MeOH	25	1415	348	1207		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	20% MeOH	25	1345	331	1147		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	10% 1-BuOH	25	132	32	112		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	7% 1-BuOH	25	211	52	179		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	5% 1-BuOH	25	428	105	364		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	5% 2-PrOH	25	1662	409	1418		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	10% 2-PrOH	25	1222	300	1042		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	15% 2-PrOH	25	759	186	647		29
C16H10	fluoranthene	Novapak C18 4 μ m	SDS	20% 2-PrOH	25	688	169	586		29
C16H10	pyrene	Supelcosil LC-18	Brij 30	60% ACN		62	29	81		14
C16H10	pyrene	Novapak C18 - 4 μ m	Brij-35		25	52	54	46	107	30
C16H10	pyrene	Hypersil C18 - 5 μ m	Brij-35			302	320	272	276	28
C16H10	pyrene	Spherisorb C8 - 5 μ m	CTAB	3% n-BuOH	25	3226	1174	3222	1291	8
C16H10	pyrene	Spherisorb C8 - 5 μ m	CTAB	5% n-BuOH	25	271	98	270	109	8

compound	name	stationary phase	surfactant	additive % v/v	T °C	P_{WM}	K_{AM} or K_2 (L/mol)	K_{AM} or K_2 (mL/g)	ϕP_{WS} or K_{AS}	ref.
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	5% MeOH	25	140200	34503	119679		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	10% MeOH	25	142587	35076	121666		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	15% MeOH	25	86338	21239	73670		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	20% MeOH	25	35430	8716	30231		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	724	178	617		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	486	119	414		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	286	70	243		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	7838	1928	6687		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	6437	1583	5492		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	5460	1343	4658		29
C20H12	benzo(a)pyrene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	4669	1148	3983		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	Brij-35		25	640	680	577	1400	30
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	5% MeOH	40	84967	30928	84849		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	10% MeOH	40	27766	10106	27727		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	15% MeOH	40	13299	4841	13280		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	24603	8955	24568		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	13666	4974	13646		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	8655	3150	8642		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	7075	2575	7065		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	5486	1996	5477		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	4355	1585	4348		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS		25	1500000	1	3	3400000	30
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	5% MeOH	25	33214	8170	28340		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	10% MeOH	25	17887	4400	15262		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	15% MeOH	25	15028	3697	12822		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	20% MeOH	25	11864	2918	10123		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	373	92	318		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	1080	265	921		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	1860	457	1587		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	8119	1997	6927		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	6055	1489	5166		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	5256	1293	4484		29
C20H12	benzo(b)fluoranthene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	4808	1182	4102		29
C20H12	benzo(e)pyrene	Novapak C18 - 4 μ m	Brij-35		25	236	250	212	460	30
C20H12	benzo(e)pyrene	Hypersil C18 5 μ m	Brij-35			217	230	195	213	28
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	5% MeOH	40	90413	32910	90288		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	10% MeOH	40	30405	11067	30362		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	15% MeOH	40	15146	5513	15124		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	27968	10180	27929		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	16210	5900	16187		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	10260	3734	10245		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	7536	2743	7525		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	5764	2098	5755		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	4766	1734	4758		29
C20H12	benzo(e)pyrene	Novapak C18 - 4 μ m	SDS		25	569107	140000	486016	1200000	30
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	5% MeOH	25	17170	4224	14650		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	10% MeOH	25	14970	3682	12772		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	15% MeOH	25	13351	3284	11391		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	20% MeOH	25	12186	2997	10397		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	597	147	508		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	748	184	637		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	1191	293	1015		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	7399	1820	6313		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	4319	1062	3685		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	4360	1072	3720		29
C20H12	benzo(e)pyrene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	2377	584	2027		29
C20H12	perylene	Supelcosil LC-18	Brij 30	60% ACN		93	44	121		14
C20H12	perylene	Novapack C18 4 μ m	Brij-35		25	524	557	473	983	30
C20H12	perylene	Novapack C18 4 μ m	CTAB	5% MeOH	40	149069	54261	148864		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	10% MeOH	40	39169	14257	39114		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
C20H12	perylene	Novapack C18 4 μ m	CTAB	15% MeOH	40	31030	11295	30987		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	3% 2-PrOH	40	48809	17766	48741		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	5% 2-PrOH	40	25078	9128	25043		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	10% 2-PrOH	40	20204	7354	20176		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	5% 1-BuOH	40	14236	5182	14216		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	7% 1-BuOH	40	13107	4771	13088		29
C20H12	perylene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	7024	2556	7013		29
C20H12	perylene	RP-18 (Waters) - 10 μ m	SDS		25	1178863	290000	1006748	670000	18
C20H12	perylene	Novapak C18 - 4 μ m	SDS		25	1138212	280000	972033	2400000	30
C20H12	perylene	Novapack C18 4 μ m	SDS	5% MeOH	25	184205	45314	157177		29
C20H12	perylene	Novapack C18 4 μ m	SDS	10% MeOH	25	40198	9888	34299		29
C20H12	perylene	Novapack C18 4 μ m	SDS	15% MeOH	25	38967	9586	33249		29
C20H12	perylene	Novapack C18 4 μ m	SDS	20% MeOH	25	38140	9382	32543		29
C20H12	perylene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	653	160	556		29
C20H12	perylene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	1470	361	1253		29
C20H12	perylene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	1594	392	1360		29
C20H12	perylene	Novapack C18 4 μ m	SDS	5% 2-PrOH	25	8683	2136	7408		29
C20H12	perylene	Novapack C18 4 μ m	SDS	10% 2-PrOH	25	7316	1799	6241		29
C20H12	perylene	Novapack C18 4 μ m	SDS	15% 2-PrOH	25	5289	1301	4512		29
C20H12	perylene	Novapack C18 4 μ m	SDS	20% 2-PrOH	25	5191	1277	4429		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C20H12	perylene	Supelcosil LC-18	Tween 60	60% ACN		137	170	129		14
C20H22NO6	dihydropyridine ester 17	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	232	84	231	88	31
C20H22NO6		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	430	105	365	109	31
C20H22NO6	dihydropyridine ester 4	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	108	39	107	34	31
C20H22NO6	dihydropyridine ester 4	Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	291	71	246	46	31
C20H22NO6	dihydropyridine ester 5	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	171	62	170	68	31
C20H22NO6	dihydropyridine ester 5	Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	643	158	549	156	31
C20H24NO6	dihydropyridine ester 10	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	136	49	134	41	31
C20H24NO6		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	408	100	347	78	31
C20H24NO6	dihydropyridine ester 11	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	141	51	140	46	31
C20H24NO6		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	416	102	354	82	31
C20H24NO6	dihydropyridine ester 15	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	133	48	132	38	31
C20H24NO6		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	359	88	305	57	31
C20H24NO6	dihydropyridine ester 16	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	122	44	121	36	31
C20H24NO6		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	359	88	305	56	31
C20H24NO7	dihydropyridine ester 18	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	81	29	80	15	31
C20H24NO7		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	314	77	267	19	31
C21H22N2O4S	dansyl-phenylalanine	nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	46	11	39	24	5
C21H22N2O5S	dansyl-tyrosine	nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	188	46	161	377	5
C21H24N2O7S	dihydropyridine ester 23	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	122	44	121	37	31
C21H24N2O7S		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	721	177	614	91	31
C21H24NO6	dihydropyridine ester 7	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	226	82	225	103	31
C21H24NO6	dihydropyridine ester 7	Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	432	106	368	145	31

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	Φ P _{WS} or K _{AS}	ref.
C21H26NO7	dihydropyridine ester 19	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	92	33	91	23	31
C21H26NO7		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	367	90	312	37	31
C21H38CIN	cetylpyridinium chloride	Hypersil - 5 μ m	CTAB		amb	2300	837	2297	250	24
C21H38CIN		CPS Hypersil - 5 μ m	CTAB		amb	4300	1565	4295	910	24
C21H38CIN	cetylpyridinium chloride	SAS Hypersil - 5 μ m	CTAB		amb	3000	1092	2996	850	24
C21H38CIN		MOS Hypersil - 5 μ m	CTAB		amb	4700	1710	4694	1000	24
C21H38CIN	cetylpyridinium chloride	ODS Hypersil - 5 μ m	CTAB		amb	2900	1055	2896	610	24
C21H38CIN		SAS Hypersil - 5 μ m	CTAB		25	3023	1100	3019	1000	25
C21H38CIN	cetylpyridinium chloride	SAS Hypersil - 5 μ m	CTAB	NaCl -0.1M	25	963	350	961	400	25
C21H38CIN		SAS Hypersil - 5 μ m	CTAB	5% MeOH	25	2089	760	2086	500	25
C21H38CIN	cetylpyridinium chloride	ODS Hypersil - 5 μ m	CTAB		25	2932	1067	2928	667	25
C21H38CIN		ODS Hypersil - 5 μ m	CTAB	NaCl -0.1M	25	3930	1430	3925	1000	25
C21H38CIN	cetylpyridinium chloride	ODS Hypersil - 5 μ m	CTAB	5% MeOH	25	2171	790	2168	500	25
C22H12	benzo(ghi)perylene	Novapak C18 - 4 μ m	Brij-35		25	247	262	222	511	30
C22H12	benzo(ghi)perylene	Novapak C18 - 4 μ m	CTAB		40	512	186	510	341	30
C22H14	dibenzo(ac)anthracene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	17270	6286	17245		29
C22H14	dibenzo(ac)anthracene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	721	177	614		29
C22H14	dibenzo(ac)anthracene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	3708	912	3163		29

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ΦP_{WS} or K _{AS}	ref.
C22H14	dibenzo(ac)anthracene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	5366	1320	4578		29
C22H14	dibenzo(ah)anthracene	Novapak C18 - 4 μ m	Brij-35		25	242	256	217	634	30
C22H14	dibenzo(ah)anthracene	Hypersil C18 5 μ m	Brij-35			133	140	119	132	28
C22H26NO6	dihydropyridine ester 14	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	254	92	252	135	31
C22H26NO6		Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	456	112	389	200	31
C22H26NO7	dihydropyridine ester 6	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	144	52	143	55	31
C22H26NO7	dihydropyridine ester 6	Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	424	104	361	88	31
C22H26NO8	dihydropyridine ester 3	Spherisorb ODS-2 - 5 μ m	CTAB	5% 1-BuOH	30	50	18	49	14	31
C22H26NO8	dihydropyridine ester 3	Spherisorb ODS-2 - 5 μ m	SDS	5% 1-BuOH	30	249	61	212	23	31
C23H13	benzo(ghi)perylene	Hypersil C18 5 μ m	Brij-35			245	260	221	242	28
C23H13	benzo(ghi)perylene	Novapack C18 4 μ m	CTAB	10% 1-BuOH	40	46000	16781	46038		29
C23H13	benzo(ghi)perylene	Novapack C18 4 μ m	SDS	10% 1-BuOH	25	730	180	625		29
C23H13	benzo(ghi)perylene	Novapack C18 4 μ m	SDS	7% 1-BuOH	25	2800	695	2410		29
C23H13	benzo(ghi)perylene	Novapack C18 4 μ m	SDS	5% 1-BuOH	25	5000	1232	4275		29
C23H18N2O6	dihydropyridine ester 1	Spherisorb ODS-2 - 5 μ m	CTAB	% n-PrOH	30	108	39	107	32	31
C23H18N2O6	dihydropyridine ester 1	Spherisorb ODS-2 - 5 μ m	SDS	% n-PrOH	30	391	96	333	59	31
C23H23N3O4S	dansyl-tryptophan	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	53	13	45	20	5
C27H29N3O4	phe-phe-phe	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	83	20	71	174	5
C30H36N4O6S2	didansyl-lysine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	82	20	70	55	5
C33H33N3O7S2	didansyl-tyrosine	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	157	38	134	253	5
C36H38N4O5	phe-phe-phe-phe	Nucleosil C18 5 μ m	SDS	3% 2-PrOH	40	179	44	153	920	5

compound	name	stationary phase	surfactant	additive % v/v	T °C	P _{WM}	K _{AM} or K ₂ (L/mol)	K _{AM} or K ₂ (mL/g)	ϕP_{WS} or K _{AS}	ref.
NO2-	ion nitrite	Spherisorb ODS - 5 μ m	CTACl			2929	916	2861		1
NO3-	ion nitrate	Spherisorb ODS - 5 μ m	CTACl			2397	750	2341		1

Surfactants : SDS= sodium dodecylsulfate; CTAB = cetyl trimethylammonium bromide; Brij® 35 = polyoxyethylene 23 dodecyl ether or C12E23; Brij®30 = polyoxy ethylene 4 dodecyl ether or C12E4; DOSS = dioctylsulfosuccinate de sodium or Aerosol OT; MB-14 = myristyl (C14) betaine; SB-12 = dodecyl dimethyl (3-sulfopropyl) ammonium hydroxyde; THPA = tetraheptyl bromide (a nonmicelle forming surfactant); Tween® 60 = polyoxyethylene 20 sorbitan monostearate. For the numbered dihydropyridine esters, see Ref. 31.

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Appendix IV

How to Prepare a Ternary Phase Diagram

Micellar solutions simply are binary solutions of surfactant in water. However, very often a ternary component – an organic modifier – is added. The physicochemistry of a ternary system is commonly studied referring to its ternary phase diagram. Numerous diagrams are published in the literature. However, it is likely that the particular phase diagram needed in a MLC separation will not be found. It is not difficult to prepare a ternary phase diagram in less than a day's work. However, the procedure is rarely taught to students in analytical chemistry. Therefore it is presented here.

Any composition of a system with three components, A, B and C, can be represented in two dimensions if a relation is made between the A, B and C proportions. The relation is:

$$A\% + B\% + C\% = 100\%$$

The percentage can be volume %, mole % or, the easiest to measure, mass %. Figure 2.15 shows how any given composition can be represented in an equilateral triangle.

To prepare the ternary diagram in mass, four mixtures of B and C will be considered. They are 80% B + 20% C, 60% B + 40% C, 40% B + 60% C and 20% B + 80 % C. In each mixture, small amounts of A are

added to the B + C mixture. Let us consider an actual example: we were unable to find in the literature the water/heptane/sodium dioctyl-sulfosuccinate (AOT) ternary diagram. To obtain the phase diagram presented by Figure 2.16, we prepared a mixture of 80% heptane and 20% AOT. Actually 0.8 g (1.17 mL) of heptane was introduced in a 30-mL test tube closed by a rubber cap to avoid evaporation. 0.2 g of AOT (a wax) was added and fully dissolved in heptane. This composition corresponds to 80% heptane + 20% AOT near the lower right corner of Figure 2.16. Next, the test tube and its holder are put on a balance that is zeroed. The cap is opened and a drop of water is added. The balance indicated 0.064 g. Shaking the tube made the water drop disappear and form a clear solution. The composition is calculated as followed: mass of heptane = 0.8 g, mass of AOT = 0.2 g, mass of water = 0.064 g, total mass = 1.064 g. The mass percentages are : heptane = 75.19%, AOT = 18.80% and water = 6%. This composition is located on a line starting from the 80-20 heptane-AOT initial composition and going to the water apex at the lower left of the triangle. By visual observation, the homogeneous water solution is referred to as a L2 system (Chapter 2).

A second drop of water was added to the mixture, the balance read 0.108 g. The heptane and AOT masses were unchanged. The total mass was 1.108 g, the water mass was 0.108 g or 9.75% w/w. After shaking, the second drop solubilized in the heptane phase. We were still in the L2 area. More drops of water were added and the test tube was weighted and shaken in a similar way. It is observed that the water dissolution takes more and more time and shaking energy. When the balance read 0.592 g, a cloudy heptane mixture was obtained. After more shaking, the cloudy solution remained. The limit of the L2 zone were was reached. The composition was: total mass = 1.592 g, heptane = 50.25%; AOT = 12.56% and water = 37.19%. Continuing the water addition in the test tube, a viscous cloudy solution was obtained for a water mass of 0.728 g. When placing the test tube between a polarizer and crossed analyzer glasses, some light could be seen. The viscous cloudy liquid was a heterogeneous system containing a liquid crystal suspended in an L2 solution (dotted area in Figure 2.16). The viscous mixture became fluid again for a water mass of 1.506 g (water = 60% w/w). Letting the test tube stand for 2 min showed a phase separation. Water was further added up to fill the test tube. The final water mass was 25 g giving a biphasic system containing 96.15% of water, 3.08% of heptane and 0.77% of AOT.

The same procedure was followed starting with a test tube containing 0.6 g (0.88 mL) of heptane and 0.4 g of AOT (line 60-40% w/w). Water was added dropwise and Table IV.1 lists the observed phenomena. Once the 40-60 and 20-80 lines were diluted the same way, the respective L2, L1 and liquid crystal areas became almost drafted. The same procedure was followed starting with water + AOT mixtures diluted by heptane. Then, special mixtures in the water rich corner were prepared to exactly delineate the L1 and liquid crystal areas.

Table IV.1 Observations Diluting the 60%-40% Heptane-AOT Mixture with Water.

Water mass g	Total mass g	Water %	Heptane %	AOT %	Observation
0	1.000	0	60	40	initial 1g mixture
0.050	1.050	4.76	57.14	38.10	clear and fluid L2 phase
0.100	1.100	9.09	54.55	36.36	L2 phase
0.150	1.150	13.04	52.17	34.78	L2 phase
0.200	1.200	16.67	50.00	33.33	L2 phase
0.300	1.300	23.08	46.15	30.77	L2 phase
0.550	1.550	35.48	38.71	25.81	L2 phase
0.900	1.900	47.37	31.58	21.05	L2 phase
1.223	2.223	55.01	26.99	18.00	mixture of L2 phase and liquid crystal
1.634	2.634	62.03	22.78	15.19	true liquid crystal
2.337	3.337	70.03	17.98	11.99	cloudy biphasic system
3.359	4.359	77.06	13.76	9.18	clear and fluid L1 phase
4.276	5.276	81.05	11.37	7.58	cloudy fluid phase
18.00	19.00	94.74	3.16	2.10	lightly cloudy fluid phase

When more than three components are needed, two dimensional representation is still possible if two relationships are imposed on the system. For example, Figure 2.18 shows phase diagrams of water, oil, SDS and alcohol. The constant molar ratio 2 SDS molecules for 13 alcohol molecules was imposed onto the system.

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